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1917

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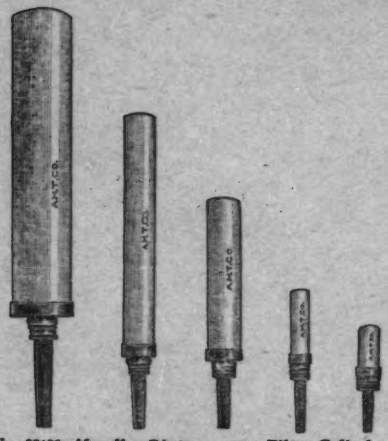

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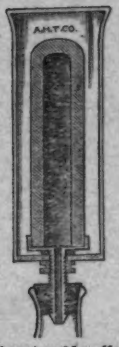

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THE EXCITATION OF MICROSCOPIC AREAS: A NON-POLARIZABLE CAPILLARY ELECTRODE

FREDERICK H. PRATT

From the Physiological Laboratory of the Medical Department, University of Buffalo

Received for publication February 12, 1917

In the study of the response of tissues presenting an aggregate of contractile elements, it has been necessary for the most part to interpret the activity of the unit in the light of data furnished by the whole. This is especially true of skeletal muscle, where the observer is hampered in the functional isolation of fibers through the mechanical influence exerted by each upon its neighbors.

Whereas the diffuse stimulation of functionally independent cells, such as melanophores (1), may yield information strictly relative to the cell under consideration, the most convincing results in the field of the muscle fiber permit only of inference from the behavior of reduced aggregates (2), (3), (4). It would seem important that the study of fiber-activity be carried beyond the realm of even the clearest deduction into that of direct observation. Any vestige of doubt attaching to the validity of the all-or-none principle, or to the adequacy of our conceptions of the genesis of tetanus, the factors of fatigue, etc., cannot but delay approach to the ultimate problem, that of the energy transformation within and about the sarcomere. It is evident that such direct observation can be attained only through a means of excitation that shall affect a contractile area of no greater diameter than that of the element under scrutiny.

In a series of experiments made in this laboratory during the last three years, designed primarily to amplify the evidence—so masterfully

established by the work of Keith Lucas—for the all-or-none principle in skeletal muscle, a form of electrode has been evolved which renders the delicately controlled excitation of exceedingly minute superficial areas possible over long periods of time, without injury to the tissue.

Credit is due to Mr. John P. Eisenberger, undergraduate assistant in physiology, for aid in the construction of the apparatus, and for essential contributions to the method of preparing the capillary pore.

CONDITIONS FULFILLED IN THE ELECTRODE

In order to apply a controlled electrical stimulus to one of an adjacent number of contractile units, such as the superficial fibers of a muscle, the following requirements are to be met:

(1) The device should be unipolar in action.

(2) The active electrode must be of less diameter than one functional unit, yet must not involve excessive resistance.

(3) Contact between electrodes and tissue must cause no mechanical or other injury, and must be uniform in tension.

(4) The electrodes must be of the non-polarizable type.

The author has made considerable use of microscopically pointed metallic electrodes, in many cases with useful results; but in no instance can all of the above conditions be fulfilled. Platinum may with some difficulty, copper with great ease, be sharpened to the requisite tenuity. Moreover a light, uniform contact may be attained by embedding the point in a globule of glass or sealing-wax, and permitting it to rest upon the surface of the preparation by gravity. It is extremely difficult, however, to keep such an electrode clean without marring the point; and its consecutive use is short lived, owing doubtless to electrolytic and polarization effects.

In the electrode here described, the active pole is formed by a physiological solution (NaCl or Ringer's) contained in a glass tube, the converging lumen of which ends in a pore which is of distinctly less diameter than the frog's sartorius fiber. This pore is thus at the apex of a conical liquid conductor of relatively low resistance, bringing into contact with the tissue a minutely circumscribed area of the solution.

The indifferent pole is that portion of a like solution which lies in contact with the tissue at the opening of an annular orifice formed by the junction of two concentric glass tubes, the inner of which, described above, contains the fluid of the active electrode and ends below in the pore (fig. 1).

Both electrodal orifices lie in nearly the same plane; they are separated by a surface of polished glass, which may be kept at uniform tension against the surface of the preparation by an appropriate adjustment.

The fluid of each pole is led through glass tubing to a bath of like solution, in which lies a porous cup (conveniently a porcelain "boot-electrode") containing saturated zinc-sulphate solution. An amalgamated zinc rod, lying in this, forms one terminal of the stimulating circuit (fig. 3).

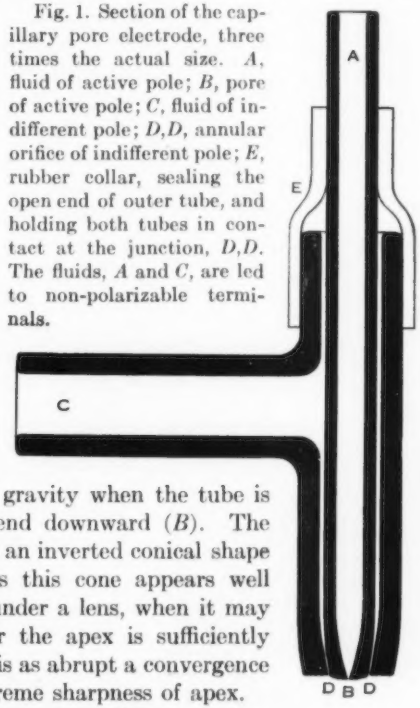

Fig. 1. Section of the capillary pore electrode, three times the actual size. *A*, fluid of active pole; *B*, pore of active pole; *C*, fluid of indifferent pole; *D,D*, annular orifice of indifferent pole; *E*, rubber collar, sealing the open end of outer tube, and holding both tubes in contact at the junction, *D,D*. The fluids, *A* and *C*, are led to non-polarizable terminals.

PREPARATION OF THE ELECTRODE

The active electrode tube is made from glass tubing of about 3.5 mm. outside, and 2 mm. inside diameter. One end is heated in the flame of the blast lamp until closed by fusion (fig. 2, *A*). Heating is then continued until the viscous end of the tube elongates slightly under the influence of gravity when the tube is held vertically with the fused end downward (*B*). The lumen will now tend to assume an inverted conical shape at its lower end. As soon as this cone appears well marked it should be examined under a lens, when it may readily be determined whether the apex is sufficiently sharp. The form to be sought is as abrupt a convergence as possible, consistent with extreme sharpness of apex.

The closed end of the tube is now ground flat on a carborundum wheel, until the apex of the lumen is within a millimeter of the surface. Grinding is continued on fine carborundum cloth until the apex is nearly reached (*C*), when crocus cloth is substituted as an abrasive. The polishing is completed on a well-worn area of the crocus cloth. Here the greatest caution is necessary to avoid undue encroachment upon the apex. Frequent examination with a powerful lens is essential, and the appearance of the slightest speck in the center of the sur-

face should call for especially critical scrutiny. A bright point of light appearing when the tube is held for examination with the open end toward the source of illumination, indicates the attainment of the apex (fig. 4). The orifice may, however, have become plugged as soon as produced, in which case it is necessary to resort to the method of clearance described later (page 165). The process of polishing is laborious and time-consuming, but due care yields a pore of extremely small size, quite circular in form, opening abruptly upon a slightly convex surface unmarred by scratches. The tube may now be cut to the requisite length.

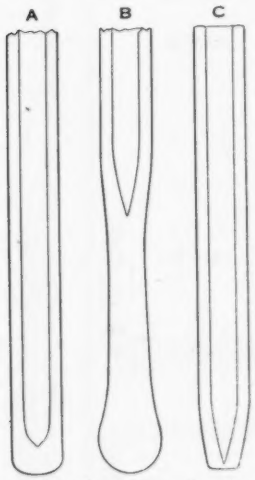

Fig. 2. Stages in the preparation of the capillary pore. *A*, tube closed by fusion; *B*, appearance of the conical extremity of the lumen when slightly elongated by gravity; *C*, after grinding. For the completed tube, see figure 1.

For the indifferent electrode a small T-tube is selected, into the main piece of which the tube just completed will glide snugly without binding. One end of the main piece is constricted slightly by heat, and ground flat. A ground joint is now made between the inner and outer tubes, with the aid of fine emery, so that the periphery of the pore-bearing surface is flush with the encircling rim (fig. 1, *D*, *B*, *D*).

When the inner tube is fixed rigidly in position by means of a coupling of rubber tubing (fig. 1, *E*), the electrode is in position for use when filled and connected.

ARRANGEMENT OF ACCESSORY PARTS

As shown in figure 3, the introduction of the electrode into the circuit involves simply a continuation of the inner tube and the branch of the T-tube, each to a bath of the physiological solution employed in the experiment. The vertical arms of the connecting tubes, just before entering the liquid, are supported by soft rubber clips attached to a horizontal rod common to both. This rod is actuated by rack and pinion for horizontal, and by worm and gear for vertical adjustment. This device (omitted from the figure) permits of delicate and uniform approximation of the electrode to the surface of the tissue. Each porous "boot" is held against the side of the glass container by a rubber band. The zinc rods, lying in the zinc-sulphate solution, have the usual connection with the stimulating circuit.

The live preparation—in most cases a frog of medium size—is entirely enclosed in a moist chamber consisting of a glass or composition dish with ground edges, covered by a plate of glass pierced with a circular orifice to admit the end of the electrode.

The large mechanical stage, carrying the moist chamber with the entire electrodal system and its adjustments, is made by extending the carriage of a stage of ordinary size by means of a glass plate, supporting the overhanging weight with a steel ball-bearing. This permits of

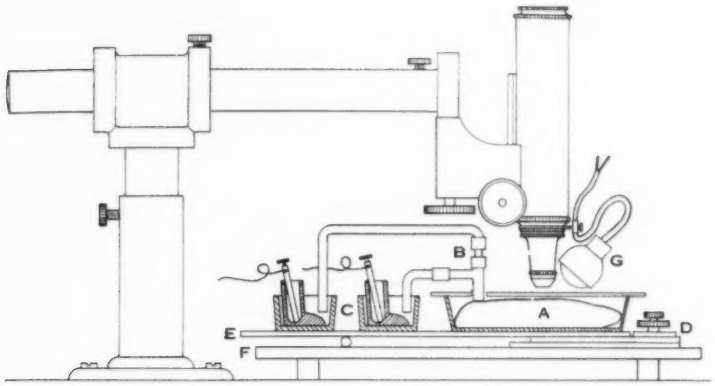

Fig. 3. Semi-diagrammatic elevation and section, showing the arrangement of accessory apparatus. The device for adjusting the electrode is omitted for clearness. *A*, live preparation, contained in the moist chamber, covered by a glass plate perforated to receive the electrode; *B*, the electrode, each fluid pole continuous with its bath, *C*, in which lies the porous "boot" containing $ZnSO_4$-Zn terminal; *D*, mechanical stage, extended by the glass carriage, *E*, which is supported upon a ball-bearing moving over the heavy plate-glass base, *F*; *G*, electric bulb, illuminating the surface of the preparation.

sufficiently frictionless movement on the part of the stage extension over the fixed base. As the contraction of single muscle fibers are best observed at a distance from the point stimulated, this arrangement enables the contracting element to be readily found, and adjusted with great nicety in the microscopic field. The figure illustrates a very rigid stand for the microscope, made from a set of ordinary steam connections. It should have horizontal swivel and forward and back adjustments, all delicate control being cared for by the mechanical stage. It is merely a convenient substitute for the expensive large dissecting stand, and on comparison has been found equally reliable for this work.

Illumination is afforded by a small electric bulb, attached by means of stout but readily flexible wires to the tube of the microscope. It should furnish an amount of heat just sufficient to maintain the temperature of the inner surface of the cover-glass above the dew-point.

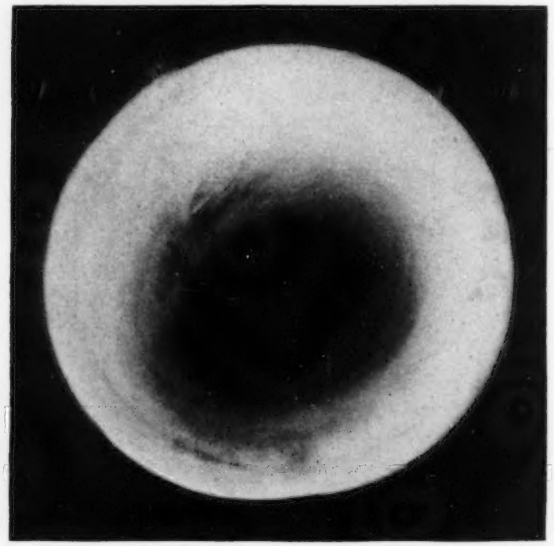

Fig. 4. Face of the active electrode, magnified 30 diameters and photographed by transmitted light. The pore, 8 μ in actual diameter, appears as a bright point in the center.

PROCEDURE IN THE USE OF THE APPARATUS

Since the preparation, so far in practice, is fed by an intact circulation, it has been found advisable to bare the tissue and enclose the pithed animal in the moist chamber at the outset, with the lamp adjusted and lighted. The delay will thus permit temperature and vasomotor conditions to attain equilibrium. Meanwhile the saline baths and porous cups are filled, the zinc terminals connected, and finally the electrode examined, coupled and fixed in place.

Three precautions are especially important:

(1) The pore of the active electrode must be clear.

(2) The electrodal system of tubes must be protected from siphonage while in position.

(3) Short-circuiting must be guarded against.

The pore in present use is 8μ in diameter—about that of the human erythrocyte—and consequently subject to frequent occlusion. The pore-bearing tube should be kept in filtered distilled water when not in use. Fortunately, the difficulty of maintenance is vastly less than in the case of a true capillary tube, which is impracticable for this purpose, owing to the excessive electrical resistance and liability to penetrate the tissue, as well as to inevitably serious plugging. In most cases, all that is necessary to free the pore of an occluding particle is to centrifuge the tube, previously filled, *in the direction of its open end*, by a sharp downward swing of the hand, as in replacing the mercury column of a clinical thermometer. The particle is usually carried into the abruptly diverging lumen, whence it may be washed out with a capillary pipette.

The connecting tubes may be guarded from siphoning out the contents of the electrode by stuffing them in a few places with absorbent cotton. A few air-bells may safely be ignored, provided there is continuity of fluid about them. These tubes, with the T-part of the electrode, may be kept permanently in the physiological solution, protected from evaporation.

The free end of the active electrode (fig. 1, *A*), when in use, is coupled to its connecting tube leading to the saline bath. Obviously, any continuity of electrolyte extending over the tube at the level *A*, to the fluid within the collar, *E*, must short-circuit the current. Spontaneous drying usually remedies this; but it is advisable to see that all superfluous solution is removed from the outside of this joint, and the possibility of leakage excluded, before proceeding to an experiment.

In adjusting the two parts of the electrode for use the rubber collar, *E*, is first raised slightly. Both parts being filled, the inner tube is thrust into the rubber collar under an ample bath of the solution. Both are then removed together from the bath, and the inner tube carried further downward until it seats itself firmly in the ground joint. The rubber collar is now slid downward slightly, providing a uniform elastic tension sufficient to hold the inner tube securely in its ground seat.

The connecting tubes are inserted in the clips, the electrode attached and contact with the preparation made by means of the adjustments already described. Under the conditions, the tension upon the tissue

is not a rigid one, owing to the yielding of the rubber connections and the flexibility of the light arm supporting the clips. It is subject, moreover, to delicate variation at will, and, once adjusted, is uniform.

DISCUSSION OF PRINCIPLES INVOLVED

An examination of the possible field of excitation (fig. 1, *D*, *B*, *D*), reveals an annular orifice surrounding a minute central pore, the two being electrically united by a film of liquid occupying the region of approximation with the tissue. It may be assumed that current density is relatively high at any tissue surface opposite the pore, diminishing with great rapidity as the annular orifice of the indifferent pole is approached. Current should penetrate little if at all the interior of the tissue, owing to the high resistance there to be encountered as compared with that of the electrolyte employed. Deep stimulation, therefore, is avoided, as evidenced by the fact that at no time is an experiment disturbed by nerve response in uncurarized preparations.

Local excitation of contractile elements beneath the face of the electrode must therefore be limited to a superficial region surrounding the pore, determined in area by (a) the strength of current, (b) the threshold of the elements impinged upon.

Since the mass of conducting electrolyte, and inversely its excitation-value, varies rapidly between the center and the periphery of the system, being based upon the extremes of a pore, 8 μ in diameter,[1] and an orifice, π (2550) μ in periphery (leading from the ample crevices of an annular ground-glass joint), it is unlikely that more than two elements can be excited when the stimulus at the pore-region is just liminal. In the case of the sartorius fibers, which exhibit a surface breadth of approximately 20 to 50 μ, it seems certain that a delicate adjustment of current will in the majority of instances cause an excitation of but one fiber, provided its surface be sufficiently near the pore.

The construction of the active electrode tube renders this close approximation of pore and tissue possible (necessarily allowing for the interposition of delicate fascia), owing to the convexity of the face of the electrode, produced, and in fact hardly to be avoided, by the method of grinding. The firmer contact with the tissue at the region of the pore than elsewhere would, indeed, further emphasize the conductor-difference between *B* and *D*, *D* (fig. 1).

[1] A pore of less than half this diameter has recently been produced, and it is probable that further experience will enable still smaller sizes to be made.

The ease with which perfusion may be accomplished within the lumen of the active electrode tube suggests its possible adaptation to sharply localized chemical stimulation or other chemical influence, with or without the use of the electric current.

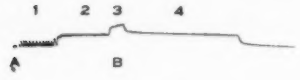

FIG. 5

FIG. 6

FIG. 7

Fig. 5. Photomicrographic record, traced by the light-reflex from a globule of mercury resting upon the active area of a sartorius with intact circulation. The plate was moved by clockwork. The stimulus, an alternating magneto current, underwent continuous increment of strength (but not of frequency) from *A* to *B;* then was maintained at full strength for the remainder of the record. Note, *1*, fiber-rhythm, often characteristic of response in the threshold region; *2*, minimal step; *3*, primary submaximal step, with contracture (treppe?); and return to *4*, minimal step, subject to contracture addition. *Continuous increment of excitation yields discontinuous increment of response. Continuous constant excitation yields discontinuous decrement of response.*

Fig. 6. Tracing obtained as in figure 5. The stimulus, in full strength at *A* suffered continuous decrement to *B*. Note the two submaximal steps and the minimal step. *Continuous decrement of excitation yields discontinuous decrement of response.*

Fig. 7. Minimal tetanus, obtained as in figure 5. Between *A* and *B* the stimulus underwent continuous increment and continuous decrement in immediate succession. The fact that the curve of relaxation is accurately superimposable upon the corresponding minimal drop in figure 5 suggests that decrement of stimulus in one case, and fatigue in the other, have eliminated the same contractile entity. This statement is made on the basis of a comparison of direct photographic enlargements five times the size of the above figures.

These curves are all from the same microsopic field and index-point, without alteration in the position of the electrode. The tracings, presenting an original magnification of 13 diameters, are enlarged by one-half and reversed to read from left to right. One and one-half millimeter on the abscissa corresponds to one second in time. The frequency of excitation was approximately 50 per second. Four hours after the adjustment of the preparation, circulation and irritability were apparently unimpaired.

An extended series of observations, shortly to be published, has indicated that the above considerations hold good in actual practice. Muscle fibers excited with tetanic stimuli glide singly or in small groups among their fellows, dragging them, passive, into positions of altered tension. The method permits readily of quantitative record—photographic, semi-mechanical, or by direct observation over the microme-

ter scale. The actual minimal response apparently reveals itself; and in clear relation to the submaximal effects elicited by increase of the area of adequate excitation. Figures 5, 6 and 7, explained by their legends, typify results regarded by the author as supporting the contention that the all-or-none law of Bowditch and of Lucas is applicable in the gradients of both tetanus and fatigue as a principle of physiological quanta.

SUMMARY

1. The desirability of a method for the individual stimulation of closely associated functionating elements is emphasized, in view of the discrepancy which is possible between unit and aggregate behavior.

2. A form of electrode is described, having an active pole 8 μ in diameter, uninjurious to delicate tissues, and non-polarizable. The active pole occupies a minute pore opening from the fused and ground extremity of a glass tube; the indifferent pole occupies the annular orifice formed by the junction of this tube with an outer tube bearing a T-arm. Each tube is filled with a physiological solution led to a porous cup containing a $ZnSO_4$-Zn terminal.

3. The method of preparing the electrode, its arrangement for use with a mechanical stage, and details relative to the use of the apparatus are described.

4. The distribution of current between the face of the electrode and the preparation is such that an exceedingly minute area may be excited without overstepping the threshold of adjacent contractile elements.

5. The adaptation of the active electrode tube to chemical stimulation is suggested.

6. Photomicrographic tracings are appended, illustrative of the method and supporting, in the author's estimation, a principle of physiological quanta for gradients in tetanus and fatigue.

BIBLIOGRAPHY

(1) Spaeth: This Journal, 1916, xli, 577.
(2) Gotch: Journ. Physiol., 1902, xxviii, 395.
(3) Lucas: Journ. Physiol., 1905, xxxiii, 125; 1909, xxxviii, 113.
(4) Mines: Journ. Physiol., 1913, xlvi, 1.

THE ACCURACY OF MOVEMENT IN THE ABSENCE OF EXCITATION FROM THE MOVING ORGAN

K. S. LASHLEY

From the Johns Hopkins University and the Government Hospital for the Insane

Received for publication February 19, 1917

For psychology the problem of motor activity has been largely one of the perception of movement. Discussion has centered about the questions of the receptors which are excited differentially by changes in extent and force of movement, about the psycho-physics of the constant error, the influence of the emotions upon the perception of movement, the relation of the "will impulse" to the perception of movement; with the result that the equally important questions of the nervous mechanism of initiation, continuation and cessation of adaptive movements have been dealt with only incidentally as throwing light upon this perception. Whether such an attitude on the part of investigators is the result of the general concept of psychology which would restrict its scope to the study of sensation, or is a consequence of the relative ease with which the problems of sensory and motor physiology may be attacked, the result has been an almost total neglect of the neurological problems presented by skilled movements.

In many cases the perception and control of movement may be one and the same phenomenon, but certain experimental data and facts of every day experience indicate a certain degree of independence of the accuracy of movement from any stimulation resulting from the movement itself. Such is the accuracy of automatic movements as seen in the production of steps of uniform length, or, more strikingly, the swift movements of the musician, many of which are made more quickly than the briefest cortical reaction time. These movements are frequently regulated in extent and force with the utmost nicety, under conditions where any reflex control from excitations arising in the moving hand seems excluded. Again, the evidence obtained by Bowditch and Southard, Loeb, Woodworth and others, while by no means conclusive, indicates a partial independence of the motor control from afferent processes originating in the moving organ. The reëstablishment of walking movements in the dog after section of the dorsal roots gives further evidence for the same view.

Clearly the ideal opportunity for the study of these relations would be given by a case of complete sensory anesthesia in man with no more impairment of motor functions than would necessarily arise from the anesthesia. A partial condition of this character is occasionally reported in tabes dorsalis and the complete absence of afferent impulses in rare cases of spinal lesion. Through the courtesy of the staff of the Government Hospital for the Insane, I have had opportunity to examine a number of patients showing various degrees of anesthesia and to carry out the experiments reported in the following pages with one who showed a condition particularly adapted to answer some of the questions centered about the control of movement.[1]

The present paper is devoted to a study of the sensory and motor condition of this patient. He is a young man of more than average intelligence who has, as a result of a gun-shot injury to the spinal cord, a partial anesthesia of both legs with motor paralysis of the muscles below the knees. The anesthesia of the left leg is much more extensive than that of the right. Rough preliminary tests indicated a sensitivity to movement of all joints except the left knee and ankle, and the paralysis of the muscles controlling the latter made it necessary to restrict the study to the movements at the knee. The experiments were arranged for the investigation first, of excitations from movements at this joint; second, of the accuracy of control of such movements as failed to excite afferent impulses from the limb.

PHYSICAL CONDITION OF THE SUBJECT

Neurological examination shows the left leg, with the greater part of the left groin and gluteal region, to be insensitive to light touch, temperature, and to protopathic stimulation. The extent of the area of cutaneous anesthesia is shown in figure 1. Except for the rather large areas in the gluteal region and the somewhat smaller areas on the calf (shown in white in figure 1) there is complete loss of cutaneous sensitivity of this leg. The loss of sensitivity to deep pressure is much less extensive, complete anesthesia to deep pressure involving only

[1] I wish to express my obligation in particular to Superintendent W. A. White, who has given me every opportunity for investigation, and to Dr. S. I. Franz, who first brought the patient to my attention and who has given important suggestions concerning the control of the experiments. Finally my greatest debt is to the subject of the present study who has worked tirelessly, and who has himself suggested improvements in technique which were essential for the success of the experiments.

the foot and anterior surface of the lower leg (cross-striated area in figure 1), but in the regions retaining sensitivity this is reduced much below normal. The minimum amount of pressure that can be detected in the region of cutaneous anesthesia is 900 grams on an area one-

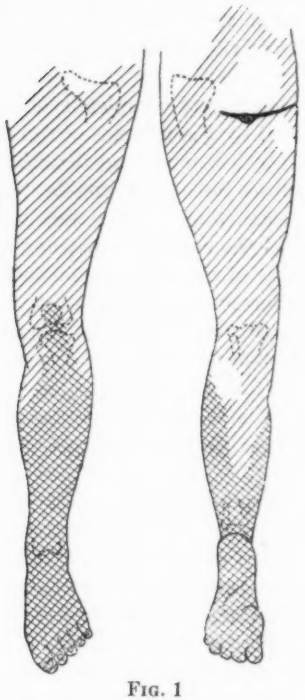

FIG. 1

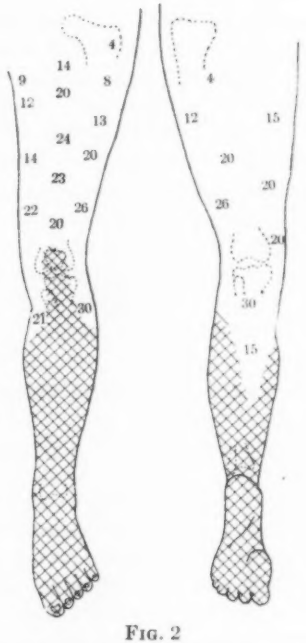

FIG. 2

Fig. 1. The extent of anesthesia of the left leg of the subject studied. White areas, cutaneous sensitivity; light shaded areas, cutaneous anesthesia with deep sensitivity to pressure; dark shaded areas, complete anesthesia to all stimulation.

Fig. 2. Threshold of sensitivity to pressure applied over an area ½ inch in diameter. Shaded area completely insensitive. The figures give the sensitivity of other regions in 100 gram units.

half inch in diameter in the region of the groin and this increases to 2000 grams over the greater part of the thigh and calf. The thresholds to deep pressure over the anesthetic area are shown in figure 2. Except in the gluteal region, in the groin and on the calf, localization

of deep pressure is very inexact, the average error of localization being about eight inches. No amount of pressure that I was able to apply excited deep pain.

There is a slight sensitivity to vibration (50 per second) in the femur. A small area of the patella also seems sensitive to this type of stimulation, but its sensitivity is probably due to the transmission of the vibration to the femur. There is no sensitivity to this type of stimulation in the region below the knee.

The cutaneous and tendon reflexes of the leg are completely abolished but a slight degree of tonicity is retained by the muscles of the thigh. We do not know the exact extent of the lesion and no detailed clinical history of the operation to remove the bullet is available, but the clinical picture indicates an extensive destruction of the dorsal bundles in the second or third lumbar segment of the cord with invasion of the dorsal horns or injury to the afferent roots in the sacral region sufficient to abolish the tendon reflexes. The determination of the exact extent of the lesion is not essential to the present work since the conclusions depend wholly upon the experimental determination of the extent of anesthesia to movement.

SENSITIVITY TO POSITION AND TO PASSIVE MOVEMENTS

Preliminary tests had indicated that in spite of the sensitivity to pressure retained by the subject, his sensitivity to flexion and extension of the knee was abolished. Before a study of the accuracy of active movements could be undertaken it was necessary to establish this point beyond question. A number of experiments was therefore carried out with a view to revealing any sensitivity to movement which might play a part in determining the accuracy of adaptive movements. In all the tests on movement the subject was seated on a soft cushion in a rather high chair with his left thigh supported by a padded rod, 3 inches behind the knee, so that his foot could swing freely from the knee through an arc of about 130 degrees from complete extension to the point where the heel came in contact with the rungs of the chair. The foot was attached by a cord to the carriage of a modified form of the Münsterberg movement apparatus, so that the rate and extent of movement could be recorded. The subject was blindfolded during all the tests and precautions were taken to eliminate any auditory stimulation from the recording apparatus which might serve as a clue to the extent of movement. The distance moved was recorded in centimeters

read directly from the recording device. A slight inaccuracy is introduced here, since the measurement was in a straight line while the movement was about the perimeter of a circle. In the records one centimeter is equal to approximately 1.28 degrees rotation about the knee.

Detection of passive movements. The experimenter grasped the subject's foot in his right hand and, holding his left hand on the subject's knee to detect unintentional movements of the thigh, flexed and extended the knee passively through angles varying from 10 to 130 degrees. The subject was asked to describe the distance and direction of movement whenever he felt that any movement had been made. The rate of the passive movements was varied from about 2 cm. to 100 cm. per second.

When the passive movements were made at a rate of less than 20 cm. per second, the subject was unable to detect any movement, however great its extent, and throughout a series of fifty such movements gave no reaction except when the movements resulted in hyperextension of the knee. More rapid movements were detected in about 50 per cent of the trials but reports of their direction and extent seemed to be wholly a matter of chance.

Fifty passive movements of the foot at a rate of more than 20 cm. per second were made under conditions where auditory cues were eliminated. The subject was asked to give the direction of movement and position of the foot whenever he felt that it was moving. Twenty-four of the movements brought no reaction. The remaining 26 were detected as movement; 4 were described as of uncertain direction, 22 brought definite statements of direction of movement which were, however, quite inaccurate. Seven movements of extension and 4 of flexion were described correctly. Five movements of extension were described as flexion and 6 movements of flexion were described as extension. The description of the position of the foot was equally inaccurate. The subject usually denied any knowledge of its position and in the instances where he ventured a definite statement he was as frequently wrong as right. Thus, in 3 out of 6 cases flexion at 70 degrees was described as complete extension and in 2 of 5 cases hyperextension was described as flexion.

When the knee was hyperextended the subject invariably stated that the knee was being moved. His knee was alternately hyperextended and flexed a number of times and he was asked to describe the movements. Only those of extension were detected and these the subject alternately stated to be extensions and flexions. Flexing move-

ments brought no reaction. From this it seems that the reactions to hyperextension were to the strain on the joint or ligaments and not to the movement as such.

An attempt was made to locate the source of stimulation by which rapid movements were detected. Slight flexion of the hip (elevation of the knee through a distance of 2 cm.) was for the first three trials interpreted as extension of the knee, thereafter as a movement of uncertain direction. Upward pressure against the patella was once interpreted as an extension of the knee, twice as a movement of the knee of uncertain direction, and remained undetected in more than 20 cases. Heavy pressure above the knee was once described as a movement of extension but later failed to elicit any response. The patient was instructed to contract the antagonistic muscles of the thigh so as to increase the tension upon the knee joint and fix the leg at the thigh. When this was done no passive movements were detected, unless they produced flexion of the hip. The quick movements which brought reactions were frequently described as "jerks" and I could not be certain that any of the movements that were detected did not involve a certain amount of strain upon the muscles of the trunk.

These tests suggest the absence of any afferent excitations from the leg during slow passive movements of the knee which may stimulate verbal reactions to the direction or extent of the movements. From them it seems probable, also, that the recognition of quick passive movements is not based directly upon excitations from the moving parts but only indirectly upon the spread of the shock of movement to adjacent parts of the body. Whatever may be the basis of the reactions to rapid passive movements the stimuli involved are not differentiated enough to allow the subject to determine the direction or extent of the movement.

Maintenance of position. The preceding tests required chiefly reaction to movement with, possibly, very little stress upon local signs from the position of the leg. A further series of tests was made with the emphasis upon position.

The subject's knee was hyperextended and he was asked to maintain that position as long as he could against the force of gravity. The quadriceps became tense and the leg was kept extended for about ten seconds, then dropped to vertical with a series of spasmodic twitches. The subject, when questioned, stated that he was still holding the leg extended and one minute later said "Now I am letting it down," the leg, in the mean time, hanging lax. The test was repeated a number of

times and gave similar results in every case. There was momentary maintenance of position, relaxation without recognition of the movement, and later an illusion of relaxation.

Other positions than that of hyperextension could not be maintained at all. When the subject's foot was placed passively in any other position and he was asked to hold it there, the leg dropped back to the vertical as soon as the support was removed, jerked back and forth spasmodically for a few seconds, and then came to rest in the vertical position, the subject asserting, without certainty, that he was holding the leg still.

Reaction to position. With the weight of the subject's foot wholly supported by the experimenter, the knee was flexed, starting from 105 degrees, until the recording carriage moved 10 cm. The subject was told that his foot would be moved back to the starting position and was asked to indicate verbally when it reached that position. The movements from the starting position were made as a uniform rate; the rate and direction of the "returning movements" were varied. The subject was warned in each case that the displacement of the foot was completed and that the experimenter was returning it to the starting point. In five series different procedures were employed: (1) The foot was moved back very slowly toward the initial position; (2) It was returned to the initial position at the same rate at which it had been displaced; (3) It was moved back very quickly; (4) The displacing movement was continued so as to bring the foot farther from the starting point; (5) The foot was held motionless after being displaced.

The positions stated by the subject to be the one from which the foot was originally displaced are given in table 1. Slow return was overestimated, quick return underestimated, and lack of movement or movement in the wrong direction was not detected. The subject stated that he was depending upon the time interval after the warning that the foot was being returned to its original position, and the relation of the positions identified as the starting point to the rate of passive movement confirms his statement. The results of the tests show clearly that there were no afferent excitations capable of arousing a reaction to position.

Duplication of passive movements. The data presented thus far indicate that any excitations from the position or from slow passive movements of the leg are subliminal for the language mechanism of the subject. It seemed possible that the excitations from passive movements

might still be above the threshold for the reflex control of active movement and to test this experiments were carried out in which the subject was asked to duplicate the extent and direction of a passive movement by making an active movement.

The position of the subject described above was used. Distances of 2 and 10 cm. were selected as pattern movements, and the subject's foot was moved backward or forward through these distances at a rate of less than 10 cm. per second. The subject stated that he could not tell when the pattern movement was made, so he was warned each time

TABLE 1

Reactions to position of the leg. A constant position of the knee (105 degrees extension) was adopted as a "starting point." The subject's foot was displaced passively 10 cm. from this and he was asked to indicate when it had been returned by a second movement. The figures, each based on the average of five trials, give the distances and the directions traversed by the foot in the second movement before the subject indicated that the starting position had been reached.

FOOT DISPLACED		EXTENT OF SECOND MOVEMENT		ERROR IN IDENTIFICATION OF POSITION	RATE OF SECOND MOVEMENT
	cm.	*cm.*		*cm.*	
Backward	10	1.4	Forward	− 8.6	Very slow
Backward	10	12.2	Forward	+ 2.2	Same as that of displacement
Backward	10	22.5	Forward	+12.5	Quick
Backward	10	0.0	Forward	−10.0	No movement
Backward	10	25.5	Backward	−35.5	Quick
Forward	10	2.1	Backward	− 7.9	Very slow
Forward	10	8.3	Backward	− 1.7	Same as that of displacement
Forward	10	16.7	Backward	+ 6.7	Quick
Forward	10	0.0	Backward	−10.0	No movement
Forward	10	8.5	Forward	−18.5	Quick

his foot was moved and was asked to continue the movement through the same distance and in the same direction as the passive movement.

Owing to the fact that subject could not hold his foot steadily in any position against the pull of gravity it was necessary to counterbalance the weight of the foot immediately before and at the expiration of his movements. Since the weight to be supported changes greatly with the degree of extension of the knee, no practicable method of supporting the weight could be employed in the limited time available exept that of partial support by the experimenter's hand. This introduces into the distances moved an element of error from the personal equation of the experimenter. On page 181 is recorded an experiment in which the same technique is used where the subject attempted to duplicate his own active

movements. In the forward movement no resistance except that of gravity was offered to the movement; in the backward movements the experimenter tried to oppose a constant resistance equal to that necessary to hold the foot in position before the beginning of the movement. The slight difference in the variability of the movements made under the two conditions of resistance indicated that the error introduced by the personal equation of the experimenter is not great enough to affect the results seriously.

The relation of the direction of the subject's movements to the direction of the pattern set is shown in table 2. The direction was interpreted wrongly in almost 50 per cent of the trials, which indicates that the direction of the subject's movements was wholly a matter of chance. The lengths of the movements varied from 0 to 27 cm. (the maximum

TABLE 2

Record of attempts to duplicate the direction and distance of passive movements. The length of the passive movements in each series was kept constant but the directions were varied in irregular order.

PASSIVE MOVEMENT	ACTIVE MOVEMENT	
Backward flexion 2 cm..... 15 trials	Flexion	6 trials
	Extension	9 trials
Extension 2 cm..... 21 trials	Flexion	7 trials
	Extension	14 trials
Flexion 10 cm..... 18 trials	Flexion	7 trials
	Extension	11 trials
Extension 10 cm..... 20 trials	Flexion	8 trials
	Extension	12 trials

Total, direction correct 39 trials
Total, direction incorrect 35 trials

movement permitted by the position of the subject's foot). The variations in two series of tests are shown in figure 3, other tests giving similar results. There is no correlation either in direction or extent between the active movements and the passive pattern which they were intended to reproduce.

The subject's failure to give any precise or constant reaction to the position or movement of his knee seems to prove that the anesthesia of the leg is sufficiently extensive to exclude any reflex control of the accuracy of movement based upon cortical excitations arising from the moving limb. The one remaining possibility of excitation from the limb calls for the postulation of receptors in the muscles which are stimulated by active contraction and not by passive tension. To

test this the relative lengths of the voluntary movements which the subject estimated as equal when different amounts of resistance were opposed to the movement were measured.

AFFERENT EXCITATION FROM THE CONTRACTING MUSCLES

Rough tests were first carried out with different amounts of resistance opposed to the subject's active movements. He was asked to flex his knee so that his foot was drawn back 3 inches from a given position (120 degrees extension) in which his leg was held by the experimenter and to indicate verbally when he had moved his foot through this distance. The latter precaution was taken to test the relation between the duration of the motor innervation and any excitation of the language mechanism which might exist.

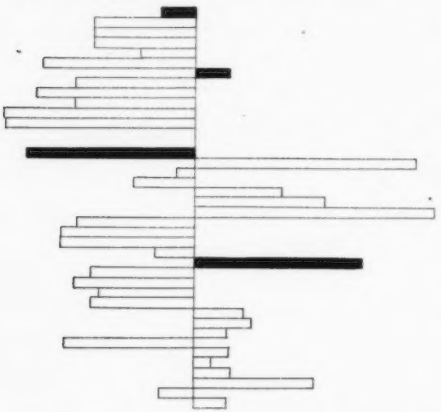

Fig. 3. Record of attempts to duplicate by active movements a pattern set by passive movement of the leg. The direction and extent of the passive movements are shown by the solid rectangles; the active movements of the subject are shown by those in outline. The pattern was repeated before each active movement.

In the first trials the foot was allowed to move at a rate of only about 1 cm. per second. The average distance moved and reported as 3 inches under this condition was 1.65 cm. with a range from 0.9 to 2.5 cm. (ten trials). In the next ten trials the pull against the subject's movements was increased to such an extent that the knee was extended slightly during his attempts to flex it. In these tests an average forward movement of 1.04 cm., with a range of 0.5 to 1.5 cm. was reported as a backward movement of 3 inches. During these tests the subject reported that he felt resistance but had increased the force of his movements until he had compensated for it completely. The mechanism by which the resistance was detected was not determined. It may have been a residual joint or tendon sensitivity which

was too slight to be stimulated by unresisted movements, or to the deep muscle sensitivity to pressure, or to certain stimulation from strain on the muscles of the trunk which could not be altogether eliminated. The subject indicated his knee as the source of stimulation, but was very uncertain. Whatever the locus of excitation, the stimulus was not specific enough to give a clue to the extent of movement of the foot. In a third series of tests the active movement of the subject was accelerated by the experimenter, the subject being asked, as before, to stop the movement and indicate when his foot had moved 3 inches. The average distance moved in ten trials was 28.94 cm. with a range from 26.0 to 30.5 cm. This was practically the maximum extent of movement allowed by the position of the subject.

TABLE 3

The average length of voluntary movements (flexion of knee) stated by the subject to be equal in extent, when the amount of resistance opposed to the movement was varied

RESISTANCE		AVERAGE DISTANCE MOVED. (TEN TRIALS)	
grams		*cm.*	
1111	Accelerating	26.0	Limit of movement
0		17.4	
266	Retarding	18.4	
644	Retarding	12.4	
866	Retarding	9.6	
1133	Retarding	8.7	
1380	Retarding	8.3	
Foot clamped		0.0	
Knee forcibly extended		2 to 10	Extension

The same tests were repeated with a better control of the amount of resistance offered to the movement, and with virtually the same results. The subject's foot was supported at 120 degrees extension by a spring exerting 100 grams for each 4.5 cm. extension and he was asked to draw his foot back three inches. The initial tension on the spring was varied from 266 to 1380 grams. In other tests the foot was held motionless, in others the knee was extended during the attempt at flexion, and finally the spring was set to flex the knee. As far as possible the subject was kept in ignorance of the procedure. He was asked to indicate verbally when he had carried out the instructions. The results of the tests are shown in table 3. The distances

moved vary inversely with the resistance encountered but not in direct ratio. The foregoing tests seem to show that there are no excitations from the actively moving limb which are specific enough to give a clue to the extent of the movement.

A certain amount of adjustment to the resistance is indicated by the fact that the extent of movement is not inversely proportional to the resistance. The work done in extending the heavier springs (computed as distance times weight lifted) is greater than that performed with the lighter springs. Such a method of considering the data is misleading, however, for it considers that no work is done unless external resistance is opposed to the movement, whereas a certain amount of work is done in the contraction of free muscle, in the stretching of the antagonistic muscles, and in overcoming the resistance at the joint. It is impossible to estimate the amount of force expended in this way, but if we assume that the work done in moving the leg without external resistance is the equivalent of lifting 50 grams for the distance moved, the total amount of work done in moving against each of the resistances recorded in the table is practically the same. There is no justification for assigning this particular value to unresisted movement, but there is also no certain evidence that there was any compensation for the different resistances encountered.

There remains the subject's statement that he felt resistance and made adequate allowance for it by giving a harder pull. How much of the apparent increase in work done was due to this cannot be determined without more data than are available at the present time upon the internal resistance to movement of muscle and joint.

Such imperfect compensation for resistance as the subject may have made is irrelevant to the problem in hand since the subject failed to distinguish the extent of movement. No difference between a flexion of 26 cm. and an extension of as much as 10 cm. was detected except in the amount of resistance encountered. The recognition of resistance was evidently not based upon any excitation which could give evidence upon the direction and extent of movement.

We may conclude that we are dealing with an anesthesia to passive and active movements of the knee which is practically complete for a rate of movement of less than 20 cm. per second within an arc of 45 degrees in each direction from the right angle. With this established it is possible to test the accuracy of voluntary movements within these limits with the certainty that the intensity and duration of the innervation involved in them are not reflexly controlled by afferent excitations from the moving limb.

THE ACCURACY OF ACTIVE MOVEMENTS

Direction of movement. In the preceding tests where active movements were requested the subject made no errors in the direction of his movements. Only twenty additional trials were given in a formal test of accuracy in the direction of active movements, in all of which the movement was made correctly. In many hundreds of voluntary movements, however, I have never seen the subject make a mistake in direction, except when he misunderstood instructions. It seems certain that the voluntary excitation of a specific group of muscles is possible in the absence of afferent excitation from it.

Extent of movement. Two somewhat different methods were used for testing the accuracy in control of extent of voluntary movements. In the first experiments the subject was asked to move his foot through a given distance (an estimated inch, 2 inches, etc.) while the experimenter gave a slight nearly constant support to the backward moving foot in order to control the inability of the relaxed quadriceps to support it against the pull of gravity. The inaccuracy of this method has been considered (page 176). The resistance was applied only to the backward movements, probably accounting for the fact that they are slightly shorter than the corresponding forward ones (tables 4 and 6), and does not influence the extent of the forward movements, which are equally accurate. When a movement was made its extent was recorded and the foot was brought back passively to its initial position, usually about 110 degrees extension. None of the subject's active movements exceeded the rate of 20 cm. per second so that the controls for slow rates of passive movement apply to all the active movements studied.

In the first experiment the subject was asked to make ten attempts to move his foot through distances which he judged to be ½, 1, 2 and 3 inches. The averages for the different distances are given in table 4. The movements were all longer than the distance asked for but there was practically no overlapping between the movements estimated as different. The pattern set by the first voluntary movement was duplicated rather accurately in later movements.

In these tests the ten trials for each distance and direction were given successively and it seemed possible that this might contribute something to the accuracy of the movement through the establishment of a rhythm of motor excitation. A series of tests was made therefore in each of which movements through distances of from ½ to 6 inches were

TABLE 4

Average distances, each based on ten trials, through which the subject moved his foot when asked to move through a distance which he judged to be that given at the left. Inches were used because he was not familiar with the metric scale

ATTEMPT TO MOVE FOOT		AVERAGE DISTANCE MOVED	
inches		*cm.*	
½	Forward	2.88 ± 0.37	Forward
1	Forward	3.86 ± 0.14	Forward
2	Forward	5.16 ± 0.50	Forward
3	Forward	13.42 ± 0.87	Forward
2	Forward (later test)	7.00 ± 0.27	Forward
½	Backward	1.46 ± 0.25	Backward
1	Backward	3.40 ± 0.24	Backward
2	Backward	7.34 ± 0.68	Backward
3	Backward	11.17 ± 0.57	Backward

made successively. The results of five such tests are given in table 5. In only one of the five tests, which began with the shortest and progressed to the longest movement, was a movement shorter than the one preceding it. This case is marked in italics in the table. The subject was not told that he had made an error yet an apparent compensation appeared in the next movement, which is the longest made for that distance.

A third series of the same general character was carried out in which all the movements of a given estimated distance were made successively but the different distances to be estimated were taken in irregular order so that an estimate of the absolute distance moved rather than a

TABLE 5

Same as table 4 except that the subject attempted to estimate distances from ½ to 6 inches successively

SUBJECT ASKED TO MOVE		DISTANCE MOVED					AVERAGE
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
inches		*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
½	Forward	3.0	5.0	3.6	2.5	5.0	3.82
1	Forward	4.2	10.2	9.0	8.2	7.1	7.74
2	Forward	7.0	13.0	12.2	12.3	11.0	11.10
3	Forward	12.1	17.0	14.5	15.8	13.2	14.52
4	Forward	13.2	*13.0*	16.5	18.0	17.2	15.58
5	Forward	16.8	22.0	20.4	21.9	18.6	19.94
6	Forward	22.9	24.0	23.3	23.0	22.0	23.04

comparison of the length of successive movements was required. The order in which they were taken up was ½, 6, 1, 3 and 2 inches. The averages for the different distances with their standard deviations are given in table 6.

TABLE 6

Same as table 4 except that the different distances were estimated in irregular order

DISTANCE TO BE ESTIMATED		AVERAGE DISTANCE MOVED	STANDARD DEVIATION
inches		*cm.*	*cm.*
½	Forward	2.5 ± 0.11	0.499
1	Forward	6.5 ± 0.44	2.271
2	Forward	11.2 ± 0.71	4.357*
3	Forward	16.3 ± 1.32	6.187*
6	Forward	22.1 ± 0.56	2.633
½	Backward	2.9 ± 0.13	0.589
1	Backward	4.8 ± 0.20	1.016
2	Backward	9.3 ± 0.38	1.823
3	Backward	15.9 ± 0.41	1.929
6	Backward	20.6 ± 0.36	1.782

* The high standard deviations here are the result of one trial in each group in which the subject reported that the movement had been made when his foot did not move. It is possible that the foot caught against the floor in these cases but as this could not be verified they were included.

In all these tests there is a surprising accuracy in the extent of the voluntary movements. Too few trials were given at each distance to lend much significance to the coefficients of variation for the attempts to estimate given distances, but in every series of tests the average distances moved are roughly proportional to the distances which the subject was asked to estimate. The pattern set by voluntary movement could be duplicated with a fair degree of accuracy and the intensity of innervation could be graduated in a series of distinctly different steps.

Comparison of accuracy of movement with that of a normal subject. For the determination of the variability of movements when the subject was asked to copy a pattern set by his own active movements and for a comparison of this with the normal variability, it was necessary to eliminate any influence which the experimenter might exert in supporting the subject's foot. This was done by supporting the foot by a light spring so that the knee was partially extended. The subject was then asked to draw his foot back through a given distance and then

allow it to swing forward freely. The carriage of the recording apparatus was arranged to stop at the limit of the backward movement.

Series of twenty or more trials were obtained for estimated distances of 1, 2 and 3 inches. The average extent of movement for these three distances with the standard coefficient of variations are given in table 7, and the distribution of variations is shown in figure 4. For comparison, the results of a similar experiment on an apparently normal individual are included in the table and figure. This subject, a physician,

TABLE 7

Variation in the extent of movements estimated as equal by the anesthetic subject and by a normal individual

	DISTANCE ESTIMATED	AVERAGE EXTENT OF MOVEMENT	STANDARD COEFFICIENT OF VARIATION	ERROR FROM DISTANCE ESTIMATED	NUMBER OF TRIALS
	inches	*cm.*		*cm.*	
Anesthetic subject.....	1	4.62 ± 0.08	0.254	+ 2.12	84
	2	14.40 ± 0.26	0.122	+ 9.40	21
	3	23.98 ± 0.44	0.167	+16.48	38
Normal subject..	1	2.04 ± 0.09	0.476	− 0.46	50
	2	11.24 ± 0.21	0.193	+ 6.00	50
	3	22.30 ± 0.16	0.076	+14.80	50

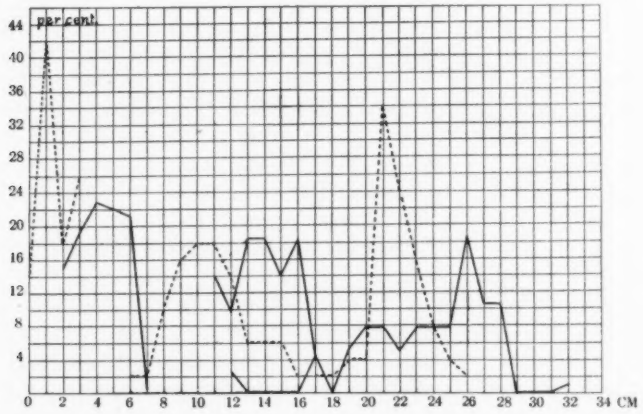

Fig. 4. Distribution of variations in the length of voluntary movements in the anesthetic and in a normal subject. The ordinates represent the percentage of the movements in each series which were of the lengths given on the abscissae. ——— Anesthetic subject.- - - - Normal subject.

was selected at random, the instructions and method of supporting the foot and recording the extent of movement were the same as those employed with the anesthetic subject so that the results with the two subjects may be taken as closely comparable. There is a surprising similarity in the results obtained with the anesthetic and with the normal subject. The movements of both showed wide errors from the distances which they were asked to estimate and the normal subject was not greatly superior to the anesthetic in this respect. In the variability of the movements estimated as equal there was no constant superiority of either subject. The normal individual gave more uniform movements for the longer distance but varied more in estimation of the shorter ones.

We may conclude that the anesthetic subject's control of his movements is not significantly less accurate than that of the normal individual, and it is not clear that for the simple movement studied the afferent impulses from the moving limb contributed anything to the accuracy of movement in the normal subject. The chief mechanism for the control of movement is located in some other body segment than that of the moving organ.

THE RELATION OF RATE TO ACCURACY OF MOVEMENT

Earlier studies of movement, particularly those of Loeb and Dellabarre have indicated that the duration of movement may serve as a clue to its extent, in place of the changing pattern of stimulation from the moving limb. It seemed possible that the subject of the present experiments was depending upon the duration of movement by maintaining a constant motor discharge during time intervals corresponding to the distances through which he was asked to move. In the following tests the rate of movement was recorded with the distance.

The subject's foot was suspended with the knee extended to 110 degrees by a spring having a coefficient of 100 grams for each 4.5 cm. extension and he was asked to draw his foot back through distances and at a rate suggested by the experimenter. The results of the tests are summarized in table 8. From this it will be seen first, that the duration of movement is not proportional to the distance when the subject is allowed to choose his own rates but that the rate of long movements is less than of the short ones (tests A, B and C); second, that the movements may be made of equal extent, although the rate is quite different (tests D and F), third, that, except in test A, the variability in the

time of movement is considerably greater than that of its extent; and fourth, that the variation in both extent and time of movement decreases with increasing rate. The experiments thus show a degree of independence in the rate and extent of movement which precludes the possibility that the extent of movement is determined merely by the control of the duration of the excitation of motor pathways. They indicate, on the contrary, that there is a control of the intensity of motor discharge which is independent both of the duration of excitation and of the effects of the discharge upon the effectors. The increase in accuracy with increased speed is in accord with the results obtained by Woodworth (6) in his study of the accuracy of automatic move-

TABLE 8

Variation of the rate of movement compared with variation in the extent of movement. In tests A, B and C the subject was allowed to select his own rate of movement; in test D he was asked to move quickly, in E, still more rapidly, and in F, to jerk his foot back as quickly as possible. The averages are each based on ten trials

TEST	AVERAGE DISTANCE	STANDARD COEFFICIENT OF VARIATION	AVERAGE TIME	STANDARD COEFFICIENT OF VARIATION	DISTANCE REQUIRED
	cm.		*seconds*		*inches*
A	4.27	0.334	0.69	0.272	1
B	8.05	0.156	2.19	0.216	2
C	11.52	0.153	3.97	0.398	3
D	12.59	0.121	1.44	0.324	3
E	9.87	0.119	0.80	0.264	3
F	11.41	0.115	0.68	0.235	3
Average		0.166		0.285	

ments and confirms his assumption that rapidity of normal movement interferes with its accuracy only by reducing the influence of the "current control," of the excitations aroused by the moving organs.

The time records showed further that the slow movements were not the result of a single muscular contraction but consisted, in practically every case, of a series of from two to five successive contractions resulting in alternate acceleration and retardation of the movement. This furnishes additional evidence against a temporal control of movement and also raises the question whether an initial set is adequate to account for an accurate movement which is excited by a series of innervations, without some controlling mechanism which is active continuously during the course of the movements.

REACTIONS TO ERROR OF MOVEMENT

In occasional instances the subject stated that a given movement was longer than he had intended. Records of only eight such movements have been obtained but in every case the recorded movement was considerably greater than other movements of the series in which it occurred. The recognition of such movements is ascribed by Woodworth (6) to sensory elements arising from the movement. If, as seems established by the tests recorded, the subject of the present experiments was anesthetic to movements of the knee, the detection of error must be ascribed to some mechanism other than the receptor system of the moving organ. This demands a distinction not only between the initial set or intention of movement and the final adjustment due to sensory stimulation, but also the recognition of a third factor in the control of movement, the capacity for reaction to the intensity of innervation which is independent of both the initial set and the excitations from the moving organ. This suggests the old doctrine of the feeling of innervation, although an alternative hypothesis must be considered. This is outlined on page 193.

EFFECTS OF FATIGUE

In some of the earlier tests, after many repetitions of a given movement, the subject complained of feeling resistance to his movements and at the same time increased their length. It seemed that this might be the result of fatigue and a number of series of movements was therefore made to test this more thoroughly. The subject was required to repeat a movement of a given length from 20 to 85 times. Resistance to the movements was offered by a spring which drew the foot back to the starting point after each movement.

Table 9 shows the results of this test. In each case repetition of the movement led to a considerable increase in its extent. The progression in the length of movement in two series is shown in figure 5 which is based upon the average of successive groups of five trials. During the later trials of the long series the subject stated that he felt tired and that it seemed to require a greater effort to move his foot than had been necessary at first.

We can scarcely interpret such data at present. The progressive increase in the length of movements estimated as equal seems almost certainly the result of the frequent repetition of the movement. From the subject's statement it seems probable also that the increase resulted

from some feeling of resistance or of increased effort necessary for the movement, which led to an over compensation. The source of the stimulation leading to this compensation offers an interesting problem. It does not seem probable that with the extensive anesthesia to all other forms of stimulation there should still persist a normal sensitivity to chemical changes in the muscles which give rise to the feeling of fatigue. The alternative seems to be some cortical mechanism by which the

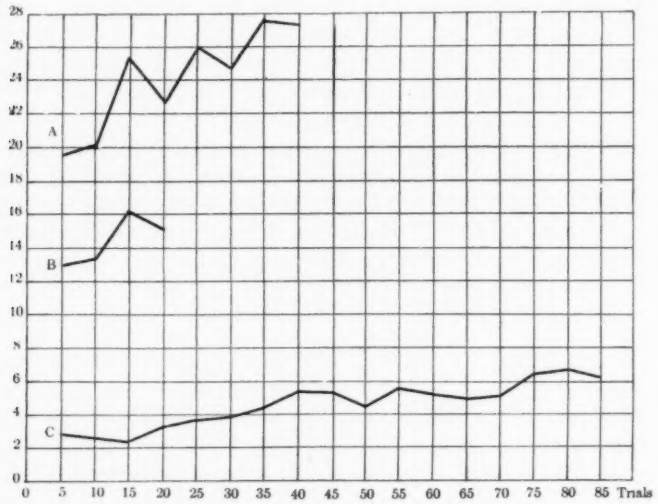

Fig. 5. The effects of fatigue upon the length of movements estimated as equal. The ordinates represent the average length in centimeters of successive movements taken in groups of five.

TABLE 9

The effects of fatigue upon the length of movements intended to be of equal extent. The subject pulled against a spring which exerted a resistance of 100 grams per 4.5 cm. extension

LENGTH OF MOVEMENT ATTEMPTED	AVERAGE EXTENT OF MOVEMENT		INCREASE	TOTAL NUMBER OF TRIALS
	First five trials	Last five trials		
inches	*cm.*	*cm.*	*per cent*	
1	2.9	6.4	120	85
2	13.0	15.1	16	20
3	19.6	27.4	39	40

increase in the threshold of excitability of the motor cells resulting from fatigue directly modifies the behavior of other action systems besides the one which is immediately involved in the movement.

THE INTERACTION OF DIFFERENT MUSCLES IN THE CONTROL OF MOVEMENT

Owing to the lack of adequate means for determining the degree of tension of the muscles of the subject's thigh, it was not possible to determine the relative functions of the flexors and extensors in controlling movement, but a few crude observations indicate that much of the normal complex interplay of the muscles is retained. In quick flexing movements a preliminary contraction of the quadriceps extensor is detectable although the inertia of the subject's foot prevents the appearance of a form of reaction movement similar to that first described by Smith (5) for finger movements. There is also, seemingly, an increase in the tension of the quadriceps as the limit of movement is approached.

When the subject is asked to contract both flexors and extensors, to "make his leg tense," an apparent fluctuation in the intensity of innervation results in oscillations of the foot, yet a given degree of extension is maintained much longer than when he is merely asked to hold his leg extended as in the experiment described on page 174. In the latter case only the extensors are in active contraction so it seems that the simultaneous excitation of both flexors and extensors permits of a steadier and longer motor discharge than is possible when only one set of muscles is innervated.

The complete loss of the tendon reflexes and the great reduction in the tone of the muscles makes a reciprocal innervation of the antagonistic muscles improbable and suggests that the interaction of antagonistic muscles in the control of movement is regulated by some part of the nervous mechanism cephalad to the spinal segment from which the muscles are innervated.

THE INFLUENCE OF TRAINING

The final question arises as to whether the condition of control of movement in this subject is comparable to that of a normal individual or whether some mechanism of control has been developed by practice which was not functional at the time of the lesion. The present observations were made five years after the spinal injury but the subject's

history scarcely supports the view that his control of movement has been reacquired by practice during this time. For the first year after injury he practiced daily walking with crutches for two hours but he made so little progress that he became discouraged and gave up all attempts to recover the lost functions, spending his time either in bed or in a wheel-chair. Except for this relatively brief practice, which aimed only at a visual control of movement, there is no history of any activity which could develop an accurate control of short, slow movements.

DISCUSSION

Every adaptive movement seems to involve three physiologically distinct processes when we attempt to analyze its neurological mechanism. These are, 1, the initiation of motor excitation resulting in muscular contraction; 2, its continuation by a series of disturbances propagated either in the central nervous system, or reflexly as a result of the motor discharge; 3, the cessation of excitation of the protagonists and excitation of the antagonists. The first of these has much in common with the simple reflex twitch and is not of moment in the problem of the control of accuracy of movement unless in some way the extent of movement may be determined by the initial excitation of the motor pathway. The continuation of the movement implies the production of a series of tetanic contractions arising from successive nerve impulses which have been excited by a single momentary stimulus. The duration of the tetanus varies with the extent of the contraction. Curiously enough, no more than vague suggestions have been advanced to account for this change from brief to long-continued excitation. The demonstration by Forbes and Gregg (2) that a single strong stimulus may induce the propagation of two or more waves of disturbance in the nerve may furnish the clue to the continuation of movement, but the increase in duration of excitation which they have shown is very slight and seems inadequate to account for movements continued for several seconds. Upon this the present work gives no data except the probable elimination of circular reflexes by which a contracting muscle might stimulate its own contraction; a possibility perhaps adequately disproved already by operative experiments.

The cessation of movement is no more explicable today than its continuation. To be useful, a movement must end with the attainment of a result which is specific for a given stimulus; it must reach a determined distance, exert a determined force, etc. In many cases the stim-

ulus to cessation or inhibition of movement evidently comes from exteroceptors and does not directly involve the receptors of the moving organs, but in the experimental duplication of a pattern set by active movement no extero-stimulation is present to determine the cessation of movement and its duration and extent must be determined wholly within the organism, either by excitation of proprioceptors or by processes carried out wholly within the central nervous system. Several alternative hypotheses to account for the cessation of movement in such cases have been formulated in such a way as to make experimental test possible.

The first of these appeals to the local sign, assuming that the extent of movement is determined by a change from one pattern of stimulation in the moving organ to another. It seems to demand a vast if not infinite series of specific reactions to different patterns of stimulation. (Ladd and Woodworth, (3) 408.)

A second assumes that the extent of movement is determined by the amount of excitation coming from the moving organ, the amount varying with the extent of movement. The hypothesis seems to demand the assumption of a priming or preliminary integration of efferent neurones before the initiation of movement.

Third, there may be purely intracortical control by some spreading of excitation, of whose nature we can form no concept at present.

Some evidence bearing upon the rôle of excitations from the moving organ in the control of accuracy has been obtained by other experimenters. Lack of space prevents any extensive summary of the literature and the thorough review of Woodworth (7) makes this unnecessary. Indications of the relative independence of motor discharge from direct control by circular reflexes come chiefly from three sources. (1) The discovery by Bowditch and Southard (1) that the reproduction by movement of a pattern distance set by visual stimulation is more accurate than that of one set by kinesthetic gives evidence of the importance of the preliminary set for accurate movement, although it throws no light upon the mechanism of the preparation for movement. (2) The observations of Loeb (4) upon the inequality of simultaneous movements of the two arms indicate that the movements of the two have a common source of control, the action of which is relatively independent of the extent of movement. (3) In his study of automatic movements Woodworth (6) found that the accuracy of movement increased in direct ratio to the speed. From this he concluded that such movements are controlled by the initial set.

The significance of the results of the present study depends upon the validity of the evidence obtained for anesthesia to slow movements. The various tests recorded have shown that: (1) The subject is unable to determine the position of his leg (except occasionally when the knee is hyperextended); (2) he can not detect the extent, duration, direction, or even the presence or absence of passive movements of the knee, if such movements are made at a rate of less than 20 cm. per second; (3) he is unable to detect the movements and changes in degree of active contraction of the muscles of the thigh occurring during his attempts to hold the knee in a given state of contraction against the pull of gravity; (4) he is unable to determine whether or not his attempts to bend his knee have resulted in movement when various amounts of resistance are opposed to the movement. In the face of this evidence we can scarcely hold that he retains a sufficient sensitivity to movement to make possible a reflex control of the extent of movement or a distinction between the extent of actual movement and the intended extent, based upon any stimulation of the receptors of the leg. We are rather forced to the conclusion that the phenomena observed are independent of afferent excitation from the moving organ.

The experiments have shown that the subject is able to control the extent of his movements with almost normal accuracy, to vary the speed and extent of movement independently, and to make rhythmic alterations of flexion and extension. The evidence for anesthesia makes it necessary to assume that all these activities may be carried out in the absence of excitation from the moving organ. The mechanism of control must be sought either in the central nervous system or in some other body segment. Data on the accuracy of movement at different rates show that in the present case its extent is not determined solely by its duration. This makes it necessary to assume some regulation of the intensity of motor discharge which is independent of its duration. Is this determined immediately by the incoming stimulus to movement resulting in a "set"[2] by which a given intensity of motor excitation is aroused explosively without further possibility of control, or is there such a spreading of the motor impulse that some control of its intensity is possible during the discharge? A certain amount of evidence bearing upon this question has been obtained. It

[2] It would be profitless to discuss here the nature or existence of attitude or set. Whether it be a priming of reflex pathways, the assumption of an altered muscular tonus, or what not, it seems distinguishable from the condition in which control of movement occurs after the initiation of the motor impulse.

comes from three sources. First, very slow movements were not made by the subject as a steady contraction of the muscles but by a series of impulses following each other at intervals of one-tenth second or more. Second, the subject was able to detect the excessively long movements of a series and to state that they were longer than he had intended to make them Third, he complained of fatigue at the same time that he showed objective signs of some disturbance in the normal conditions of movement, while chemical sensitivity to fatigue products in the muscles of the anesthetic leg seems improbable. These points are not at all firmly established by the data at hand but all indicate that there is a spreading of the motor excitation which plays a part in the control of movement and may perhaps lead to some phenomenon such as that described as the feeling of innervation. The hypothetical explanation of such a condition which is most open to experimental test is that assuming a spread of the motor impulse to other action systems with reflex control from them. It may, perhaps, be tested by a study of the possibility of controlled movements within intervals less than the minimum cortical reaction time. Experiments to this end are now in progress. As evidence for the importance of the initial set, on the contrary, there is the fact, emphasized earlier by Woodworth (6) for automatic movements, that the accuracy of movement increases directly with the rate. The evidence at hand is not adequate to rule out either alternative.

SUMMARY

Active movements of the left knee were studied in a subject having a complete anesthesia to movements of this joint. Evidence bearing upon the nature of the control of movement in such a condition suggests the following conclusions.

1. Accurate movement of a single joint is possible in the absence of all excitation from the moving organs. The interaction of the various muscles concerned in the movement is not obviously different from that found in normal subjects.

2. In contrast to reflexly controlled movements, the accuracy of movements under such conditions is in direct ratio to their rate, that is, within the limit tested, the quicker the movement the more accurately it is made.

3. The control of accuracy of the movements is relatively independent of their duration; movements of different length do not result from a uniform excitation continued for varying time intervals but from variations in the intensity of motor discharge.

4. It is probable that a control of the intensity of motor discharge after its initiation is possible in the absence of excitation from the organs activated.

5. The normal phenomena of fatigue occur when it is highly probable that the chemical sensitivity of the fatigued muscles is reduced.

BIBLIOGRAPHY

(1) Bowditch and Southard: Journ. Physiol., 1881, iii, 232–245.
(2) Forbes and Gregg: This Journal, 1915, xxxix, 172–235.
(3) Ladd and Woodworth: Elements of physiological psychology, New York, 1911.
(4) Loeb: Arch. f. d. gesammt. Physiol., 1897, xli, 107–127.
(5) Smith: Mind, 1903, xii, 47–58.
(6) Woodworth: Psych. Rev., Monograph Supplement, 1899, iii, 13, 114.
(7) Woodworth: Le mouvement, Paris, 1903.

THE CARDIO-SKELETAL QUOTIENT

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It has long been known that the strength of faradic stimulus necessary to provoke response in heart tissue is greater than that required to elicit activity in skeletal muscle. The exact relationship existing between these two tissues with respect to strength of stimulus necessary to arouse contraction has not hitherto been definitely established. Doubtless this has been due to unreliable means of measuring faradic stimuli. In the investigation of another problem in this laboratory it became necessary to make a preliminary study of the relationship existing between the threshold of the frog's ventricle and the threshold of its gastrocnemius muscle.

In this investigation the thresholds were determined by means of Martin's method of quantitative faradic stimulation (1). β units were determined in all cases. Particular care was used in selecting healthy frogs for the experiments. Each frog was pithed and weighed. The heart was exposed by a wide free incision into the anterior body wall. The pericardium was slit throughout its length and the frenum was cut. A looped silk ligature was next thrown around the heart. Posteriorly it was in contact with the white crescent, anteriorly it was tied so that the aortae were included in it. In some preliminary experiments it was found that the ordinary Stannius ligature usually stopped the heart but that it would not remain stopped long enough to determine the threshold, or would start beating the moment the threshold had been reached. If in tying the ligature the aortae were included, the heart remained quiescent for a time sufficient for the determination of the threshold. The electrodes used were the ordinary platinum electrodes of the Harvard Apparatus Company. The points were sharpened and adjusted 2 mm. apart. After the heart ligature was tied the frog was placed under the recording apparatus. The tip of the ventricle was caught in a clip which was connected with a light heart lever writing on a slowly revolving smoked drum. Then the electrodes were thrust

directly into the ventricle about 2 mm. from the auriculo-ventricular groove. The stimulation caused by placing the electrodes was usually followed by several rhythmical contractions of the ventricle. It was noted that in the first few minutes after placing the electrodes the threshold varied considerably, but that after about fifteen minutes the response became quite uniform and remained so for an hour and frequently longer. As soon as the heart showed uniformity of response the threshold was quickly determined. The usual routine was followed of finding first the threshold with no added resistance in the secondary circuit and then finding the thresholds with 10,000, 20,000 and 30,000 ohms in the secondary circuit. The resistance of the tissue was then measured by the Kolrausch method. The heart was not moistened during the experiment since it was noted that the irritability apparently shifted rapidly with each application of solution. When necessary to prevent rapid drying, a moistened filter paper was suspended so as to surround the heart without touching it and thereby serve as a moist chamber. Usually the experiment was completed within three-fourths of an hour and during this time the response was quite constant. One heart which was not moistened after it was connected with the recording apparatus maintained a uniform irritability over a period of two hours. The experiments were all performed at a temperature of 20°C. After the heart thresholds were determined, a slit was made in the skin over the gastrocnemius muscle and the same electrodes that were used in the heart were thrust directly into the middle of the belly of the muscle. A line joining the two points of the electrodes would be at right angles to the long axis of the muscle. By this method it was found that the muscle became uniform in its response in about the same time that the heart required to become uniform in response. The least visible response of the muscle was taken as the indication of the threshold. In some experiments in which the thresholds were recorded on a smoked drum it was found to correspond closely with the visible contraction observed in the intact muscle. Following the determination of thresholds with varying resistances in the secondary circuit, the tissue resistance was measured by the method previously mentioned.

Table 1 shows the results obtained. The average for the threshold of the ventricle is seen to be 191.0 Z units. The average of the β units is 98.3. In each instance the ratio of β to Z was determined. The average is 0.50. The average threshold for the gastrocnemius muscle is 33.3 Z units, 16.1 β units. It is of interest to note that the threshold

of the gastrocnemius in this series is greater than that reported by Martin in his series of eighteen experiments (2). This is due to the fact that the experiments were not performed under comparable conditions. Martin has already called particular attention (3) to the im-

TABLE 1

Showing thresholds of heart and gastrocnemius muscle and their relationship as expressed by the cardio-skeletal quotient

EXPERIMENT NO.	HEART			GASTROCNEMIUS			CARDIO-SKELETAL QUOTIENT	
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$	Z	β
4	110.0	50.6	0.46					
10	126.8	63.6	0.50	30.0	12.4	0.41	0.23	0.19
27	134.5	54.6	0.40	12.1	4.9	0.40	0.08	0.08
12	138.2	46.5	0.33	40.2	15.2	0.37	0.29	0.32
24	138.2	81.4	0.58	29.2	17.1	0.58	0.21	0.21
26	142.1	90.5	0.63	26.1	16.3	0.62	0.18	0.18
25	147.1	70.3	0.47	26.4	13.0	0.49	0.18	0.18
28	147.1	85.5	0.58	30.0	17.1	0.57	0.20	0.20
13	151.8	57.0	0.37	40.2	12.4	0.30	0.26	0.21
8	151.8	72.3	0.47	45.0	20.9	0.46	0.29	0.28
2	160.1	85.8	0.53					
6	161.1	69.0	0.42	26.1	10.1	0.38	0.16	0.14
30	166.5	99.0	0.59					
5	187.2	112.2	0.59	32.2	14.9	0.46	0.17	0.13
7	188.0	93.0	0.49	13.5	7.0	0.52	0.07	0.07
17	195.5	69.0	0.35	32.4	11.4	0.35	0.16	0.16
1	198.7	98.0	0.49					
15	212.5	111.9	0.52	22.4	12.4	0.55	0.10	0.11
22	220.5	102.7	0.46					
3	222.0	125.2	0.55	23.0	12.2	0.53	0.10	0.10
9	246.8	110.9	0.44	67.0	34.1	0.50	0.27	0.30
18	265.9	139.8	0.52					
20	276.5	198.5	0.71	61.8	38.1	0.61	0.22	0.19
29	332.8	172.5	0.51	30.4	16.4	0.53	0.09	0.09
14	355.5	197.9	0.55	45.8	21.5	0.46	0.12	0.10
Average	191.0	98.3	0.50	33.3	16.1	0.48	0.17	0.17

portance of using similar conditions if there is a desire to duplicate the experiments of another observer. In this series the electrodes were 2 mm. apart and placed in the middle of the muscle. This was done in order that they might be used in the heart and thus provide uni-

formity in the application of the stimulus to both tissues. In Martin's series an electrode was thrust into each end of the muscle, the kathode being nearest the origin. Martin used isolated muscles, in this series the muscle was left in situ. The average β unit in this series is 16.1, in Martin's series it is 8.1. Some experiments which will be discussed later were arranged in which the technique used by Martin was employed. The average β unit was then found to be 9.0.

Attention is called to the ratio of the gastrocnemius β to Z. It is 0.48. This is practically the same as Martin found in his series of eighteen experiments upon the same muscle. Martin found the ratio of β to Z to be 0.49, and the average variation from this ratio was 15 per cent. In this series the average variation is 15.6 per cent. In the heart series the ratio of β to Z is 0.50. The average variation from this is 14 per cent. The cardiac ratio $\frac{\beta}{Z}$ and the skeletal muscle ratio $\frac{\beta}{Z}$ are evidently of the same order of magnitude and from the evidence may be considered identical.

In order to determine the relationship existing between the heart Z units (HZ), and the gastrocnemius Z units (GZ), also the heart β units (Hβ) and the gastrocnemius β units (Gβ), the ratio of GZ to HZ and the ratio of Gβ to Hβ were determined in each instance. The average of the ratio $\frac{GZ}{HZ}$ was found to be the same as the ratio $\frac{G\beta}{H\beta}$. It is 0.17. In the individual experiments it is noted that the ratios were usually equal or nearly so. This equality in the ratios GZ: HZ and Gβ: Hβ was so constant that it was thought to be indicative of accuracy in the determination of the thresholds. It seemed desirable to use some expression to signify the relationship existing between the threshold of the heart and the threshold of the gastrocnemius so the term Cardio-skeletal Quotient is suggested for that purpose. It will be used throughout this discussion to signify the ratio of the threshold of the gastrocnemius muscle to the threshold of the ventricle. Thus there is a cardio-skeletal quotient for the Z units, indicated by $\frac{GZ}{HZ}$, and a cardio-skeletal quotient for β units, indicated by $\frac{G\beta}{H\beta}$. Theoretically $\frac{GZ}{HZ}$ should equal $\frac{G\beta}{H\beta}$ and in the present series this was found to be true. The average quotient in each case is 0.17.

The cardio-skeletal quotient may serve as a means of making corrections where obviously only one determination is in error out of the four usually made. Thus if the cardio-skeletal Z quotient is 0.21, then in accordance with the evidence obtained in this series the cardio-skeletal β quotient should also equal 0.21. If in making the determinations it is found that the cardio-skeletal β quotient is widely divergent from the cardio-skeletal Z quotient, it is possible by referring to the averages of the series to determine whether the error is in the Z units or in the β units. If the error is in the β units it may be ascertained, by reference to the averages in the series, whether the error is in the heart β units or the gastrocnemius β units. Finding that the error is in the heart β units the equation $\frac{GZ}{HZ} = \frac{G\beta}{H\beta}$ may be used to determine the real value of $H\beta$. An example will serve to make the point clear. In experiment 24 in table 1, the $H\beta$ was determined by the above equation. As originally determined the units and ratios were as follows: HZ 138.2, $H\beta$ 33.0, $\frac{\beta}{Z}$ 0.23; GZ 29.2, $G\beta$ 17.1, $\frac{\beta}{Z}$ 0.58; $\frac{GZ}{HZ}$ 0.21, $\frac{G\beta}{H\beta}$ 0.51. Referring in the table to the average cardio-skeletal Z quotient and the average cardio-skeletal β quotient it will be seen that in this experiment the cardio-skeletal Z quotient is approximately that magnitude which is normally present. The cardio-skeletal β quotient however is plainly an error. Apparently there was a mistake in calculating the β units. The $G\beta$ is 17.1. Reference again to the averages of the series reveals a close correspondence in the figures. The gastrocnemius ratio $\frac{\beta}{Z}$ is seen to be 0.58. This while differing from the average by about 20 per cent may be considered within the range of error. Inspection of the heart β units and the heart ratio $\frac{\beta}{Z}$ reveals the source of the error. The heart β is 33.0. This is a variation from the average as shown by the series of 66 per cent. The heart ratio $\frac{\beta}{Z}$ is 0.23. This is 54 per cent divergent from the average as shown by the series. Obviously therefore the error is in the β units of the heart. By use of the equation $\frac{GZ}{HZ} = \frac{G\beta}{X}$, in which X is the $H\beta$ sought, it is found that the $H\beta$ is 81.4. This correction necessarily implies that the heart ratio of β to Z is always the same as the gastrocnemius ratio of β to Z. That such is the case is indicated by the table. This method of correction applies necessarily to β units.

The evidence presented here indicates that the cardio-skeletal quotient is a fairly constant quantity, the average variation being a little under 30 per cent. This apparent constancy of the cardio-skeletal quotient suggested the possibility of its utilization as a means of studying the effects of various agents upon the heart, using as an index of the effect an alteration in the average cardio-skeletal quotient. In order to test the validity of the quotient, it was decided to make some practical applications of it. Use was made of the gastrocnemius experiments in table 1. Taking each experiment the gastrocnemius Z units and β units were divided by the cardio-skeletal quotient 0.17. The results were taken to represent respectively the heart Z units and the heart β units. The following averages are the results: Z units 196.1, β units 95.1, $\frac{\beta}{Z}$ 0.48. Reference to the actual determinations in table 1 shows the very close agreement of the averages. Individual experiments revealed in many instances wide variations from the actual determinations. This method clearly would not apply to one experiment but to a series.

Another test of the validity of the quotient was made. In table 1 it will be noted that there are six experiments in which the units for the gastrocnemius were not determined or were discarded because of faulty technique. In this case the corresponding heart units were multiplied by the cardio-skeletal quotient 0.17 to determine the theoretical values of the gastrocnemius muscles. Table 2 shows the results obtained. Comparison with table 1 shows again a very close agreement between the

TABLE 2

Theoretical determinations of gastrocnemius thresholds (omitted from table 1) from the corresponding heart thresholds by means of the cardio-skeletal quotient 0.17

EXPERIMENT NO.	HEART			GASTROCNEMIUS		
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$
4	110.0	50.6	0.46	18.7	8.6	0.46
2	160.1	85.8	0.53	27.2	14.5	0.53
30	166.5	99.0	0.59	28.3	16.8	0.59
1	198.7	98.0	0.49	33.7	16.6	0.46
22	220.5	102.7	0.46	37.4	17.4	0.46
18	265.9	139.8	0.52	45.2	23.7	0.52
Average.....	186.9	95.9	0.50	31.8	16.2	0.50

average theoretical determinations in these six experiments and the average actual determinations in nineteen experiments of the series.

Evidently the cardio-skeletal quotient is a reliable guide for the determination of threshold units where the conditions of experimental procedure are comparable. In case the methods are not uniform they may be reduced to uniformity in some instances and the cardio-skeletal quotient still be applied. Thus in the series of eighteen experiments upon the frog's gastrocnemius reported by Martin the average Z unit and β unit were 50 per cent lower than the same units reported in the present series. But attention has already been called to the different technique used by Martin. In his series the average Z units was 17.1, the average β units was 8.1, the average $\frac{\beta}{Z}$ 0.49. In the technique employed in this series it required approximately six times the strength of stimulus to make the heart contract that it required to make the gastrocnemius contract. Using the technique employed by Martin it would require twelve times the strength of stimulus to make the heart contract that it required to make the gastrocnemius contract. Since by the latter method results are obtained that are 50 per cent lower than the same units obtained in the present series there would also be a lowering of the cardio-skeletal quotient by 50 per cent. Difference in the manner of application of the stimulus led to a corresponding diminution in the strength of stimulus necessary to arouse the activity of the tissue. Accordingly it was assumed that the cardio-skeletal quotient in Martin's series was 50 per cent less than the same quotient in the present series or 0.085. By use of this quotient theoretical determinations of the heart units in Martin's series of gastrocnemius thresholds were made. Thus each gastrocnemius Z unit and β unit was divided by the assumed cardio-skeletal quotient 0.085 and the results taken to represent respectively the heart Z unit and β unit. The results were as follows: average Z unit 195.0, average β unit 95.3, average $\frac{\beta}{Z}$ 0.49. Comparison with the general averages in table 1 shows a striking closeness of the results.

In order to determine if the method of obtaining thresholds in the present series accounted for the difference in the same values as revealed by Martin's series, a few experiments were arranged in which the technique was the same as Martin's. The results are shown in table 3. That the assumption in regard to lowering of the cardio-skeletal quotient because of difference in technique is tenable, is clearly shown by

the results. The heart units are of the same magnitude as those of the present series, but the gastrocnemius units are of the same order of magnitude that Martin found. The ratio $\frac{\beta}{Z}$ is somewhat higher than those of the series. This could not be accounted for except that the frogs had just begun dying rapidly and some unusual disturbance may have been present which slightly raised the ratio of β to Z. The most interesting fact is the close agreement of the determined cardio-skeletal quotient with the assumed one for Martin's experiments: 0.085 and 0.09. In table 4 is presented a summary of the results obtained where actual determinations were made, also where theoretical determinations were computed.

TABLE 3

Showing thresholds of heart and gastrocnemius muscle using Martin's technique for the gastrocnemius determinations. Relationship expressed by the cardio-skeletal quotient

EXPERIMENT	HEART			GASTROCNEMIUS			CARDIO-SKELETAL QUOTIENT	
	Z	β	$\frac{\beta}{Z}$	Z	β	$\frac{\beta}{Z}$	Z	β
A	147.0	71.6	0.48	14.9	8.3	0.56	0.10	0.11
C	152.7	83.9	0.58	16.7	9.5	0.57	0.10	0.11
B	164.5	80.5	0.48	15.7	8.6	0.54	0.09	0.10
E	191.3	99.1	0.51	17.5	9.4	0.53	0.09	0.09
G	197.8	113.7	0.51	18.0	9.6	0.53	0.09	0.08
D	204.2	116.4	0.56	16.9	9.0	0.53	0.08	0.08
F	206.4	117.7	0.58	17.7	9.2	0.51	0.08	0.07
Average.	180.5	97.5	0.53	16.7	9.0	0.53	0.09	0.09

The curious effect of removing the heart entirely from the body was shown in one experiment. It was found impossible to stop the heart by means of the ligature so it was isolated by cutting through the auricles. It stopped promptly. Upon stimulating it was found to require 2496 Z units to obtain a response. The resistance of the ventricular tissue included between the stimulating electrodes and the gastrocnemius tissue included in the same area was the same. The average of each was 1500 ohms.

The evidence presented in this series of experiments seems to justify the conclusion that the cardio-skeletal quotient is a body constant. Also this quotient may be utilized in studying the effects of various

TABLE 4

Summary of average results obtained by actual and theoretical determinations

PROCEDURE	HEART				GASTROCNEMIUS				CARDIO-SKELETAL QUOTIENT		
	No. averaged	Z	β	$\frac{\beta}{Z}$	No. averaged	Z	β	$\frac{\beta}{Z}$	No. averaged	Z	β
Actual determinations.	25	191.0	98.3	0.50	19	33.3	16.1	0.48	19	0.17	0.17
Theoretical determinations of heart units in table 1, by means of the gastrocnemius experiments and the cardio-skeletal quotient.	19	196.1	95.1	0.48							
Theoretical determinations of gastrocnemius thresholds (omitted in Table 1) by means of corresponding heart experiments and the cardio-skeletal quotient.					6	31.8	16.2	0.50			
Theoretical determinations of heart thresholds from Martin's series of gastrocnemius experiments, by means of cardio-skeletal quotient 0.085. Figures in italics are Martin's.	18	195.0	95.3	0.49	18	*17.1*	*8.1*	*0.49*	18	0.085	0.085
Actual determinations using Martin's technique with the gastrocnemius muscle.	7	180.5	97.5	0.53	7	16.7	9.0	0.53	7	0.09	0.09

agents upon the heart, and conversely it may be used to study the effects of various agents on the gastrocnemius muscle. It may also be used as a means of correcting β units when there is a series to obtain averages from.

SUMMARY

1. The threshold stimulus of the frog's ventricle as shown by this series is 191.0 Z units, 98.3 β units, and the ratio $\frac{\beta}{Z}$ is 0.50.

2. The threshold of the frog's gastrocnemius by the method used in this series is 33.3 Z units, 16.1 β units, and the ratio $\frac{\beta}{Z}$ is 0.48.

3. The cardio-skeletal quotient is defined as the ratio of the gastrocnemius threshold to the threshold of the ventricle. By the method employed in this series it is 0.17.

4. Utilization of the cardio-skeletal quotient as a means of studying conditions that affect the heart is indicated.

5. By the method described in this series it is shown that it requires six times as strong a stimulus to make the frog's heart contract as it does to make the frog's gastrocnemius contract.

BIBLIOGRAPHY

(1) MARTIN: The measurement of induction shocks, New York, 1912.
(2) MARTIN: Loc. cit., 85.
(3) MARTIN: Loc. cit., 87.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLI. The Alleged Influence of the Removal of the Salivary Glands on the Secretion of Gastric Juice

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There are many factors involved in the secretion of gastric juice. Besides the reflex or psychic factor, we have the saliva itself, (Pavlov (1)), the secretagogues of the food (Schiff, Pavlov, Bayliss and Starling), and of the pyloric mucosa (Edkins). Tarulli and Pascucci (2), report gastric secretagogues in the spleen. In addition to these Keeton and Koch (3) tested various organs for the presence of secretagogues ("gastrin") and found some positive, while others, such as the submaxillary glands, gave negative results.

The present work was carried out to determine whether or not such a hormone exists in the salivary glands (and consequently affects the secretion of gastric juice by way of the blood), as has been affirmed by some but denied by others.

Since the secretion of saliva is the initial process of the digestive act, one might expect some results on digestion, on health and on the secretion of gastric juice from extirpation of the salivary glands; but in an animal like the dog, which bolts its food, and in which the ptyalin is absent, (Carlson (4)), with the secretion of the sublingual glands strongly alkaline, and with no evidence of an adaptation of the character of the saliva to diet (Garrey (5)), the effect of the removal of the salivary glands on gastric secretion is brought into question.

Hemmeter, working on dogs, reached the conclusion that a hormone is present in the salivary glands, and that the absence of the salivary glands leads to diminished gastric secretion and peptic digestion. He did not discuss the effect of their removal on the acid secretion of the gastric juice. This depressed gastric secretion, he states, can be brought back almost to normal by feeding extracts of the salivary glands. In

his work published in Science (6), he states that he used the Pavlov pouch, while in the conclusions from his work as published in the Transactions of the American Gastro-Enterol. Assoc. (7) and Biochem. Zeitschr. (8), he states that the work was done by use of the simple gastric fistula. He adds that "in some cases there is a secretion after removal of the glands" and concludes that this is due to one of the three following factors:

(a) That the lobules of the parotid are not completely removed; (b) that the psychic secretion was not completely eliminated; (c) a possible abnormal secretion.

In the dog we found that the removal of the parotid is not, after all, such a difficult task, because it is smaller than the submaxillary and because its relation to the latter and its location over the ear always give one a good clue to its location, while the space in which it lies can be increased in size by extending the head. To eliminate the psychic secretion would appear to indicate a diminished and possibly prolonged gastric secretion, and therefore lead to an incorrect interpretation of the results. The possible presence of an abnormal secretion would somehow have to be controlled by the salivary glands.

Hemmeter's conclusions are as follows:

(1) In dogs with simple gastric fistula the extirpation of all of the salivary glands produces a marked diminution in the gastric secretion. This is also evident in the analysis of test meals drawn by the test tube from animals with intact stomachs. It is necessary to prevent psychic secretion in order to bring about the phenomenon described; (2) even in animals with intact vagi it may sometimes happen that the removal of all the salivary glands causes a decided impairment of gastric secretion, so that a causative relation between the loss of the salivary glands and the reduced proteolytic and milk coagulating power of the gastric juice appears certain, even in these cases; (3) in nine salivary dogs in whom the gastric secretion has been decidedly diminished, it is not restored to the normal by the feeding of food that has been well masticated and insalivated by other normal dogs; (4) when the gastric secretion is diminished a temporary restoration may be brought about by intravenous or peritoneal injection of extracts made from the salivary glands of normal dogs; (5) this temporary restoration of gastric secretion takes place even when the stomach is isolated from the central nervous system; (6) the presence of an exciting gastric secretion hormone formed in the salivary glands. Salivary gland extract fed directly with the food or placed into the stomach directly is not capable of exciting gastric secretion. Ground up fresh salivary glands cause approximately the same gastric secretion as an equivalent amount of ground beef in these animals.

Loevenhart and Hooker (9), working on dogs with simple gastric fistulae, tried to determine the presence or absence of a salivary hormone

by feeding extracts of the salivary glands. They assumed that the presence of such a hormone would cause an increased secretion of gastric juice in normal dogs, and since they did not obtain evidence of such increased secretion they concluded that a gastric secretory hormone is not present in the salivary glands.

Hemmeter (10) later took exception to their method and conclusions, emphasizing his findings that extracts of the salivary glands raise a depressed gastric secretion almost to normal, and that their experiments on dogs with normal gastric secretion could not be used to disprove his conclusions.

EXPERIMENTAL PROCEDURE

The method employed is based on the use of the Pavlov pouch, the secretion of which may be considered a true index of the course of the secretion in the main stomach. After allowing seven to ten days for complete recovery from the Pavlov pouch operation, the gastric juice was collected from the pouch for six to eight hours each day. The rate of gastric secretion was determined by measuring the juice secreted at intervals of one hour, beginning one hour before feeding a standard meal of lean meat. In this manner a normal secretion curve was obtained over a period of seven to ten days. For each hour the rate, peptic digestion and acidity were determined and curves plotted. After the determination of the normal secretion curve the three pairs of salivary glands were removed in one operation. This was preferred to two separate operations because we observed that a second anesthesia was prone to induce infection of the respiratory tract (distemper).

After considerable experimentation, the most satisfactory *modus operandi*, and the one finally adopted, consisted in making two incisions, one on each side, extending from the ear to the angle of the mandible, each being about $2\frac{1}{2}$ inches long.

In the dog the three glands on each side approximate each other very closely; the parotid lying over the ear, its medial portion in close relation to the submaxillary; the sublingual consisting of two parts, the aboral portion lying directly on the submaxillary gland and the oral portion further up along the duct of the submaxillary in the sublingual triangle.

The manner of collecting the gastric juice and determining the rate consisted in placing a perforated rubber tube in the pouch and collecting the secretion in a container somewhat similar to the one sketched by Keeton (11) for use on cats.

TABLE 1

Summary of observations on the gastric juice of two dogs before and after extirpation of the salivary glands. The averages are made up from 20 observations of each dog, 10 before and 10 after removal of the salivary glands

	DOG	BEFORE			AFTER		
		High	Low	Average	High	Low	Average
Rate of secretion in cubic centimeters	1	15.75	7.25	**10.33**	18.50	7.00	**10.00**
	2	26.25	11.50	**17.39**	23.50	12.25	**19.33**
Total acidity in per cent	1	0.0931	0.0639	**0.0761**	0.2263	0.0712	**0.1204**
	2	0.2327	0.0626	**0.1325**	0.2934	0.1384	**0.2381**
Free acidity in per cent	1	none	none	**none**	0.1733	none	**0.0536**
	2	0.1900	none	**0.0718**	0.2489	0.0791	**0.1819**
Pepsin concentration in millimeters (Mett).	1	18.25	14.75	**13.99**	20.75	11.50	**13.96**
	2	18.75	9.75	**13.61**	25.50	14.75	**17.21**

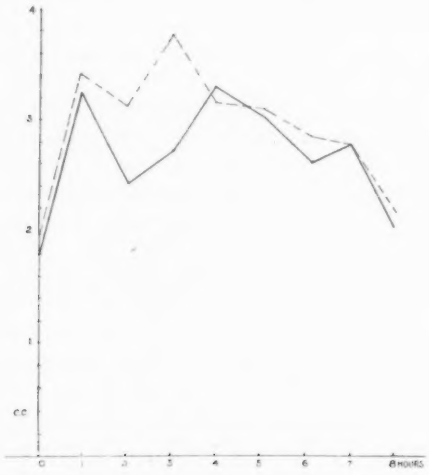

Fig. 1. Represents the rate of gastric secretion on a standard meal of meat as determined by 20 observations on a dog with a Pavlov stomach, the continuous line indicating the average of 10 observations before removal of the salivary glands, while the broken line indicates the average of 10 observations after removal of the salivary glands.

The peptic digestion for a period of twenty-four hours was determined according to Mett as modified by Cobb (12).

The free acidity was calculated by titrating 1 cc. of the gastric juice diluted with 20 cc. of distilled water with N/40th NaOH using dimethylamidoazobenzol as an indicator for the free acidity and phenolphthalein as an indicator for the total acidity.

Two vigorous dogs survived all the operative procedures, their wounds healed perfectly and their health did not seem to be at all impaired.

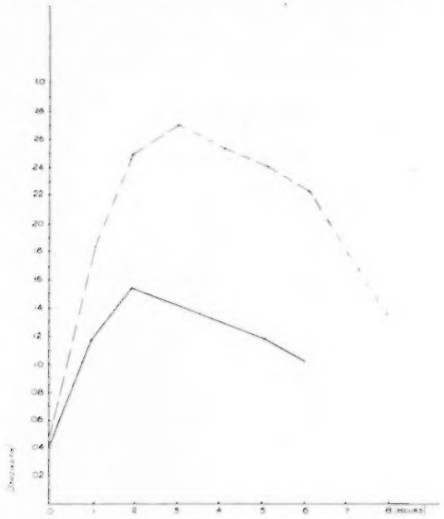

Fig. 2. Dog. 2. Represents the total acidity of the gastric juice as obtained by the average of 20 experiments. The continuous line indicates the total acidity as determined by 10 observations before the removal of the salivary glands. The broken line indicates the total acidity for a series of 10 observations after the removal of the salivary glands.

Their mouths did not appear as dry as would be expected after loss of all the salivary glands, which is probably due to the numerous mucous glands present in the oral cavity. The dogs soon learned to swallow their food and their taste did not appear to be altered.

RESULTS

The rate or quantity of secretion of gastric juice in both dogs was not altered by complete removal of the salivary glands (fig. 1).

The acidity of the gastric juice shows a greater variation, there being a decided increase after the removal of the salivary glands (figs. 2 and 3). In dog 1 before removal of the glands there was at no time any free acidity, while out of thirteen days following there was only one day in which free acid was absent. In dog 2 free acid was present both before and after removal of the glands, but the free acidity after removal of the salivary glands showed a marked increase. The maximum total acidity in both dogs occurred, on the average, half an hour to one hour

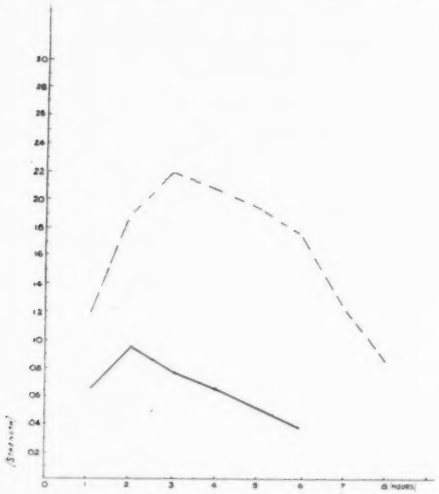

Fig. 3. Dog. 2. Represents the free acidity of the gastric juice as obtained by the average of 20 experiments. The continuous line indicates the free acidity as determined by 10 observations before the removal of the salivary glands. The broken line indicates the free acidity for 10 observations after the removal of the salivary glands.

later than in the control periods, and the subsequent fall in acidity was more gradual.

The peptic digestion was about the same both before and after the removal of the glands in dog 1, but in dog 2 there was a slight increase.

CONCLUSIONS

1. Our results contradict the theory of a hormone in the salivary glands stimulating the secretion of gastric juice. Extirpation of the

salivary glands in the dog does not decrease the gastric juice secretion (appetite and secretagogue juice).

2. Extirpation of the salivary glands causes a distinct rise in the acidity of the gastric juice. This increase in acidity is greater than can be accounted for by the slight increase in the rate of secretion. The slight increase in quantity may be due to the absence of the alkaline saliva.

3. After extirpation of the salivary glands, the maximum secretion rate after a meal appears slightly retarded. This may be due to the absence of the water of the saliva, and to decreased psychic secretion, owing to the dryness of the mouth and consequent impaired taste.

BIBLIOGRAPHY

(1) Pavlov: The work of the digestive glands (transl. by W. H. Thompson), 1910.
(2) Tarulli and Pascucci: Cited from Luciani's Human physiology (English transl. by F. A. Welby), 1913, ii, 175.
(3) Keeton and Koch: This Journal, 1915, xxxvi, 353.
(4) Carlson and Crittenden: Proc. Soc. Exper. Biol. Med., 1910, vii, 52.
(5) Garrey: Journ. Biol. Chem., 1907, iii, 40 and 41.
(6) Hemmeter: Sci., 1907, xxvi, 473.
(7) Hemmeter: Trans. Amer. Gastro-Enterol. Assoc., 1908, 5.
(8) Hemmeter: Biochem. Zeitschr., 1908, xi, 238.
(9) Loevenhart and Hooker: Proc. Soc. Exper. Biol. Med., 1908, v, 114
(10) Hemmeter: Proc. Soc. Exper. Biol. Med., 1909, vi, 33.
(11) Keeton: This Journal, 1914, xxxiii, 25.
(12) Cobb: This Journal, 1905, xiii, 448.

THE COMPOSITION OF SALIVA IN RELATION TO THE INCIDENCE OF DENTAL CARIES[1]

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INTRODUCTION

It has been previously reported by the writer (4) that the ratio of the neutralizing powers, or the power to maintain neutrality, of normal resting saliva and of the activated saliva, obtained by the chewing of paraffine, bears a definite relationship to the incidence of dental caries. In persons with carious teeth this ratio, expressed as a percentage, exceeds 80 while in persons whose teeth are temporarily immune from (or, more correctly, resistant to) caries the ratio falls below 80. In other words, as the difference between normal resting saliva and activated diminishes so does the liability to the incidence of caries increase. Shepard and Gies, in discussing this relationship or "salivary factor" have maintained (5) that it is inconstant. They based their conclusions, however, upon data which included all the different types of stimuli indiscriminately without regard to the nervous impulses and reflexes produced by the sense of taste. In their experiments they used paraffine, sucrose, sodium chlorid, alcohol and certain combinations of these. It was subsequently stated by the writer (8) that comparisons of saliva, the samples of which have been collected under different conditions, are inadmissible since such procedure ignores entirely psychic influences. Recalculation of their figures confirms the findings originally reported (4).

In a second communication (6) the writer presented data which both substantiated and developed the above thesis. Reports were made of investigations conducted in some of the state institutions for the in-

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sane, and consisted of analyses of saliva from certain cases of dementia praecox and epilepsy. This work was likewise criticised by Gies (7) and answered in turn by the writer (9). No data have been presented which disprove any of the conclusions drawn in either paper (4 and 6) and it is in the further development of the consequences arising out of these conclusions that the following experiments were undertaken.

There are two main questions which fall under consideration in this connection, namely, first, the origin of the change of the neutralizing power of saliva which occurs in response to certain stimuli, and second, the significance of this change in relation to the incidence of dental caries. In other words, whether the alteration of the salivary factor is a contributing cause of dental caries, or conversely, an effect produced by dental caries, and lastly, whether there is a cause common to both altered factor and dental caries in which case the factor would become merely an incidental symptom.

In connection with the first of these problems, I have sought to throw light on the origin of the differences between the neutralizing powers of different samples of saliva: (a) By dialysis experiments in which the attempt has been made to determine the relative magnitudes of the parts played by the inorganic, or at least the diffusible substances, and the non-diffusible, and presumably organic, constituents of saliva. (b) By the determination of the amino nitrogen content of various samples of saliva after hydrolysis with a view to estimating more exactly the part played by protein in contributing to the difference in properties and composition between normal resting and activated salivas. In connection with the second problem I have sought to extend the observations of Pickerill upon the relationship of diet and habit to the incidence of dental caries and furthermore to determine the influence of the locality of the stimulus upon the neutralizing power of the secretion which is evoked.

Part 1

The origin of the differences in neutralizing powers displayed by normal resting and paraffine activated saliva

a. The relative magnitude of the parts played by diffusible and non-diffusible substances in determining the neutralizing power of saliva

The experiments were carried out as follows: Saliva was titrated and a second set of samples was dialyzed for a period of time; then the liquids both outside and inside the membrane were separately titrated.

The dialysis of the samples was made in these cases where a sufficient quantity of saliva could be obtained without conscious exertion on the part of the patient. Following the method of C'ausen (10) and Porter (11) a solution of gun cotton in an ether alcohol mixture was made. From this solution collodion thimbles were fabricated and then placed in recently boiled distilled water until ready for use. From 2 to 5 cc. of the sample were measured directly into the thimble and then placed in a small prescription vial. Boiled distilled water was pipetted into the bottle until the level of the sample exactly coincided with that of the water. Decomposition of the sample was prevented by adding

TABLE 1

Comparison of undialyzed and dialyzed saliva

NUMBER	RESTING SALIVA								ACTIVATED SALIVA							
	Resting saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Activated saliva before dialysis	After dialysis outside tube	Inside tube	Total dialyzed	Before dialysis	After dialysis outside tube	Inside tube	Total dialyzed
	Cubic centimeters of HCl				Cubic centimeters of NaOH				Cubic centimeters of HCl				Cubic centimeters of NaOH			
E 4	6.60	3.00	2.00	5.00	13.75	9.20	4.20	13.40	24.15	16.00	2.65	18.65	4.75	2.85	1.65	4.50
E 1	9.00	6.20	5.40	11.60	9.50	8.25	0.20	8.45	17.75	14.00	0.95	14.95	5.50	4.40	0.69	5.09
E 6	19.50	12.30	1.28	13.58	6.50	6.00		6.00	48.30	46.50	3.40	49.90	1.20			
E 7	8.75	6.50	0.00	6.50	9.80	10.70		10.70	25.20	21.40	2.40	23.80	4.55	3.40	1.00	4.40
E 9	10.70	8.10	1.00	9.10	7.30	4.85	0.85	5.70	15.80	14.45	1.15	15.60	3.10	3.00		3.00
E10	14.00	9.25	3.15	12.40	5.00	4.90	0.75	5.65	16.35	13.70	2.00	15.70	4.80	2.10	2.25	4.35
E11	17.45	10.25	7.00	17.25	7.40	3.30	0.90	4.20	19.10	18.30	3.00	21.30	2.90	1.75	1.00	2.75
E12	7.25	4.00	3.15	7.15	11.75	8.10	2.70	10.80	19.90	16.70	2.20	18.90	4.85	3.10	0.95	4.05
E13	8.50	4.85	3.20	8.05	12.40	9.60	3.00	12.60	22.70	20.10	1.50	21.60	5.40	4.00	0.90	4.90
E14	16.40	9.70	5.80	15.50	8.90	4.80	3.90	8.70	35.40	23.10	3.40	26.50	1.70	1.00		1.00

one drop of chloroform and one drop of xylol. The bottle was tightly corked and placed in an air tight cabinet for twenty-four hours, at a temperature between 20° and 24°C. At the end of this time the liquid outside the membrane was titrated separately from that inside the membrane. Theoretically, if there were no loss of CO_2, from the sample, the sum of the two titration figures for either alkalinity or acidity should equal the original titration value. But a slight precipitation of phosphates, which is always evident, demonstrates that some loss of CO_2 has occurred. Data based on twelve, thirty-six and forty-eight hour dialyses showed a wider variation than those based on the twenty-

four hour limit and this later time, consequently, was chosen as a standard.

The results of this work are reported in tables 1 and 2. The analyses, although of questionable quantitative value demonstrate, qualitatively, that the greater percentage of alkalinity and acidity is found in that portion of the sample which has dialyzed through the membrane and is due, therefore, to inorganic constituents. With subject No. E4 the alkalinity of 10 cc. of the resting saliva was 6.60 cc. N/200 HCl. After dialysis the alkalinity outside of the membrane was 3.00 cc. and inside the membrane, 2.00 cc. The activated sample exhibited a

TABLE 2

Dialysable proportion of neutralizing power

NUMBER	CONDITION OF MOUTH	NORMAL RESTING SALIVA			PARAFFINE ACTIVATED SALIVA			
		Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Neutralizing power before dialysis	Neutralizing power in dialysate	Dialysable proportion of neutralizing power in percentage	Salivary factor
E 4	Immune	20.35	18.40	90.01	28.90	23.15	80.10	70.41
E 1	Immune	18.50	20.05	108.38	23.25	20.04	86.19	79.57
E 6	Immune	26.00	18.58	75.31	47.10	49.90	105.94	55.20
E 7	Immune	18.45	17.20	93.22	29.75	28.20	94.79	62.02
E 9	Carious	18.00	14.80	82.03	18.90	18.60	98.41	95.23
E10	Carious	19.00	18.05	95.00	20.15	20.05	99.50	94.29
E11	Carious	24.85	21.45	86.11	22.00	24.05	109.32	113.00
E12	Immune	19.00	17.95	94.47	24.75	22.95	92.77	76.76
E13	Immune	20.90	20.65	98.80	28.10	26.50	94.31	74.37
E14	Immune	25.30	23.20	91.70	37.10	27.50	74.12	68.20

marked difference for the titration figure of the undialyzed sample was 24.15 and for the dialyzed, 16.00 outside the membrane and only 2.65 inside the membrane. The acidities likewise show the same phenomena, the undialyzed normal resting saliva having a reading of 13.75 cc. N/200 NaOH and the dialyzed 9.20 ouside the thimble and 4.20 inside.

b. The amino-nitrogen yielded by hydrolysis of normal resting and activated saliva

In the utilization of the Van Slyke apparatus for the determination of the amino-nitrogen in the saliva the author has employed a method

which combines accuracy with simplicity. The wide application which this apparatus has found in blood analysis recommends it favorably to the problem at hand. The procedure outlined in Hawk (14) was followed with a few modifications. The technique of the analytical work was performed by Mr. S. A. Waksman and I take pleasure in acknowledging his service.

It was at first thought best to analyze the samples as soon as they were obtained from the patient but this procedure is open to objection on account of the fact that there is so small an amount of gas evolved in the reaction that accurate readings of the gas volume are exceedingly difficult. Since it has been the custom in the salivary work to secure the material between eight and eleven in the morning it was found inconvenient to make the determinations at the same time. To overcome these objections all the samples were hydrolyzed. Ten cubic centimeters of well mixed saliva were measured into a special digestion tube and 4 cc. of concentrated HCl were added. The tube employed was of soft glass about nine inches long and one inch wide. One end was sealed off and the other drawn out until the diameter was reduced to nearly $\frac{1}{4}$ inch. After the introduction of the sample and the addition of the acid the small end was sealed. These tubes closed at both ends were placed in a water bath and digested at 100°C. for four hours. Attempts to digest the samples by boiling over a flame and using a reflux condenser were unsatisfactory as bumping and loss of the material could not be controlled.

Having prepared the Van Slyke micro-apparatus in the usual manner 2 cc. of the well mixed hydrolyzed sample were carefully transferred to the measuring tube and run into the decomposing bulb. The bulb was shaken for ten minutes and the NO absorbed by the permanganate solution. The volume of nitrogen was read and the room temperature and barometer noted. Duplicate determinations were made and, in many instances, triplicates.

The results of the analyses are reported in tables 3 and 4 and represent the cubic centimeters of amino nitrogen at standard pressure and temperature yielded by 10 cc. of sample. This calculation was made so that the data would be comparable to those of the titration experiments. Inspection of the table shows that, in the greater percentage of those cases which were classed as immune from dental caries, the nitrogen content of the normal resting saliva is appreciably higher than that of the paraffine activated saliva. Those samples, however, taken

from the mouths in which caries existed did not exhibit the same difference. These facts substantiate the work done on the dialysis experiments. For it was shown that the increase of total neutralizing power in paraffine saliva from immune cases was due to a larger amount of inorganic constituents. These later tests demonstrate that a smaller percentage of organic bodies, represented by the amino nitrogen values, is contained in this same type of saliva and that therefore the difference in titration equivalents must be due to inorganic material.

TABLE 3

Caries

DATE	SERIAL NUMBER	NORMAL RESTING, CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA CUBIC CENTIMETERS OF AMINO N PER 10 CC. SAMPLE
September 18, 1916	G 2	2.20	3.00	
September 21, 1916	G 2	2.50	3.00	
September 25, 1916	G 8	3.70	3.80	
October 27, 1916	G28	3.70	3.60	
October 27, 1916	G29	3.70	3.60	
October 27, 1916	G31	4.30	4.30	
November 2, 1916	G36	4.10	3.60	
November 2, 1916	G37	4.20	3.80	
September 25, 1916	G 9	3.10	3.30	
October 5, 1916	G 9	3.00	3.00	
October 30, 1916	G 9	3.40	2.80	
September 29, 1916	G10	3.30	3.40	3.80
November 8, 1916	G10	4.15	4.27	
November 10, 1916	G10	3.77	3.63	
November 10, 1916	G18	2.80	2.95	
November 7, 1916	G18	3.10	3.20	
November 9, 1916	G18	4.10	4.00	
November 4, 1916	G38	3.90	3.80	
November 5, 1916	G42	4.20	4.10	
November 8, 1916	G44	4.40	4.50	
November 15, 1916	G 9	4.27	3.70	
November 20, 1916	G 9	5.80	3.57	
November 23, 1916	G10	4.02	4.02	
November 2, 1916	G35	4.10	4.00	
November 4, 1916	G43	5.90	4.87	3.90
November 21, 1916	G52	4.48	4.84	
November 21, 1916	G53	3.80	4.10	
November 23, 1916	G58	3.12	3.39	

TABLE 4

Immunity

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
September 16, 1916	G 1	2.40	2.30	2.20
September 19, 1916	G 1	3.20	2.90	
September 19, 1916	G 3	4.60	3.80	3.50
September 26, 1916	G 3	5.70	3.30	
September 21, 1916	G 4	3.00	2.60	
September 28, 1916	G 4	3.50	4.00	3.20
October 9, 1916	G 4	3.80	3.20	3.40
September 21, 1916	G 5	2.90	2.70	
September 21, 1916	G 5	3.20	3.00	
September 28, 1916	G 5	3.70	3.90	4.60
October 9, 1916	G 5	3.60	3.00	2.90
October 3, 1916	G 6	3.40	2.60	
October 3, 1916	G11	3.40	3.10	
October 9, 1916	G11	4.90	4.10	
October 3, 1916	G12	5.00	4.10	
October 9, 1916	G12	4.20	3.80	
October 9, 1916	G14	4.00	3.40	
October 9, 1916	G15	4.20	3.20	
October 13, 1916	G16	3.90	3.70	
October 13, 1916	G17	4.60	4.00	
October 20, 1916	G19	2.90	2.30	
October 20, 1916	G20	3.60	3.40	
October 20, 1916	G21	3.50	2.70	
October 20, 1916	G22	4.20	3.40	
October 20, 1916	G23	4.50	3.40	
October 23, 1916	G24	4.10	3.70	
October 25, 1916	G24	4.20	3.90	
October 27, 1916	G24	4.10	4.00	
October 25, 1916	G25	5.60	2.90	
October 25, 1916	G26	5.60	3.00	
October 28, 1916	G26	4.30	3.60	
October 28, 1916	G32	5.10	4.30	
October 28, 1916	G33	5.60	4.50	
October 28, 1916	G34	4.30	3.80	
November 2, 1916	G34	4.20	3.90	
November 18, 1916	G26	5.80	4.10	
November 11, 1916	G26	5.75	4.00	
November 4, 1916	G39	4.80	4.10	
November 4, 1916	G40	4.60	4.15	
November 4, 1916	G41	5.70	4.05	
November 11, 1916	G45	4.87	4.63	

TABLE 4—Continued

DATE	SERIAL NUMBER	NORMAL RESTING SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	PARAFFINE ACTIVATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE	ELECTRICALLY STIMULATED SALIVA, CC. OF AMINO N IN 10 CC. SAMPLE
November 11, 1916	G46	4.80	4.00	
November 11, 1916	G47	6.24	4.37	
November 11, 1916	G48	4.97	3.97	
November 13, 1916	G48	4.63	4.05	
November 14, 1916	G48	4.70	4.13	
November 15, 1916	G48	4.90	4.06	
November 16, 1916	G48	5.04	4.20	
November 17, 1916	G48	3.51	3.30	
November 23, 1916	G25	5.25	3.10	
November 24, 1916	G25	5.44	3.19	
November 24, 1916	G26	3.80	3.20	
November 13, 1916	G26	5.80	4.10	
November 15, 1916	G26	5.75	4.00	
November 6, 1916	G49	4.97	4.14	
November 13, 1916	G49	4.87	4.14	
November 14, 1916	G50	5.50	4.06	
November 17, 1917	G50	5.21	3.99	
December 4, 1916	G55	5.93	3.67	
December 11, 1916	G55	5.70	3.67	

On averaging these tables, the following figures are obtained:

Immunity normal resting saliva	4.47 cc. amino N
Immunity paraffine activated saliva	3.63 cc. amino N
Difference	0.84
Equals	20.7 per cent of the mean
Caries normal resting saliva	3.89 cc. amino N
Caries paraffine activated saliva	3.72 cc. amino N
Difference	0.17
Equals	4.5 per cent of the mean

The difference in nitrogen content between the normal resting saliva in cases of immunity and that in caries is strikingly brought out by the above tabulation. In the determination of averages however there is always an error which must be measured before the data may be considered conclusive.

The determination of the standard deviation of the mean demonstrates, however, the reliability of the results presented. These calculations were evaluated from the formula given by Davenport (15) as follows:

$$\text{Standard deviation} = \sqrt{\frac{\text{sum of the squares of the deviation from the mean}}{\text{number of measurements,}}}$$

$$\text{Probable error of the mean} = 0.6745 \times \sqrt{\frac{\text{standard deviation}}{\text{number of measurements.}}}$$

The appended table thus summarizes the data:

	CARIES	IMMUNITY
Standard deviation normal resting saliva.....	0.645	0.913
Probable error.....	0.08	0.07
Equals.....	2.1 per cent of the mean	1.8 per cent
Standard deviation paraffine saliva.....	0.5564	0.569
Probable error.....	0.0735	0.0496
Equals.....	2.0 per cent of the mean	1.4 per cent

It is evident from these figures that the difference between the yields of amino nitrogen by normal resting and activated saliva in persons afflicted with dental caries is only twice the probable error of the mean; in other words, that there is either no difference, or at the most only a slight one between the average protein content of normal resting saliva and activated saliva secreted by such persons. In normal individuals, on the contrary, the difference between the amino nitrogen yields is no less than ten times the probable error of the determination and it is evident that in such persons there is a very definite divergence of composition between normal resting and activated saliva. The normal resting saliva of a person with caries approaches, in protein content, the activated saliva of a normal person, and stimulation by chewing paraffine results in little change either in protein content or in neutralizing power of the saliva secreted.

These relations are illustrated in the appended table showing the average neutralizing powers (that is the number of cubic centimeters of N/200 acid and alkali required to change 10 cc. of saliva from neutrality to phenolphthalein to neutrality to paranitrophenol) of normal resting and activated saliva in normal persons and in persons afflicted with caries. These averages are compiled from salivary analyses completed within the last two years and comprise data obtained from over one hundred individuals. It is evident that saliva from subjects with

carious teeth presents two distinct differences from saliva of immune subjects, namely, that the neutralizing power of the resting saliva is supernormal while that of the paraffine activated saliva is subnormal.

Average total neutralizing power

	NORMAL RESTING SALIVA	PARAFFINE ACTIVATED SALIVA	AVERAGE SALIVARY FACTOR
Immunity	23.693	38.324	61.82
Caries	30.096	30.952	97.24

The normal resting saliva of persons with carious teeth is, therefore, characterized by (1) a relatively high neutralizing power and therefore, presumably, (2) a high content of diffusible substances; (3) a low content of proteins.

TABLE 5

Comparison of values of amino nitrogen with the total neutralizing power

NUMBER	NORMAL RESTING SALIVA				PARAFFINE ACTIVATED SALIVA				
	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	HCl	NaOH	Total neutralizing power	Amino N per 10 cc. sample	Salivary factor
	cc.	cc.		cc.	cc.	cc.		cc.	
G24	17.10	5.00	22.10	4.10	38.50	1.00	39.50	3.70	55.95
G26	27.40	4.70	32.10	5.60	58.45	−1.50	56.95	3.15	56.40
G26	20.00	4.50	24.50	4.30	49.00	0.50	49.50	3.60	49.50
G27	15.00	12.90	27.90	8.00	41.10	1.75	42.85	4.90	65.10
G 9	27.75	2.55	30.30	3.40	36.55	1.50	38.05	2.80	79.60
G45	28.50	−1.60	26.90	4.87	57.60	−4.15	53.45	4.63	50.30
G48	34.75	2.15	36.90	4.63	47.55	2.50	45.05	4.05	81.90
G48	24.00	3.30	37.30	4.90	35.00	0.60	35.60	4.06	76.70
G49	16.10	5.70	21.80	4.97	35.50	1.25	36.75	4.14	59.30
G40	18.40	3.70	22.10	5.50	31.60	2.30	33.90	4.06	65.20
G52	33.00	8.00	41.00	4.48	40.65	1.00	41.65	4.84	98.45
G53	24.15	3.95	28.10	3.80	30.80	2.80	33.60	4.10	83.60
G58	7.70	8.00	15.70	3.12	32.45	1.30	33.75	3.39	46.50
G58	11.90	6.00	17.90	4.10	33.45	1.25	34.70	3.70	51.60
G59	24.55	2.75	27.30	3.19	26.70	0.90	27.60	3.00	98.90

The inter-relation between the amino nitrogen and the salivary factor is shown in table 5. Subjects 52 and 59 were classed as carious, the others as immune. This connection of the one to the other is rendered all the more striking when the results of the dialysis experiments

are kept in mind, for the data evaluated in table 1 pointed to the fact that the increase of the total neutralizing power is due primarily to an increased amount of inorganic substances. Conversely, the lowered amount of organic bodies in activated salivas coupled with their greater neutralizing power brings further evidence to substantiate this conclusion.

From these results it is evident that immune persons secrete in response to stimulation by chewing tasteless substances a saliva which has a greater neutralizing power than normal resting saliva and is furthermore differentiated from normal resting saliva by a considerably lower content of protein and higher content of inorganic salts. The alteration in the character of the saliva is not merely due to dilution, consequent upon more rapid secretion, but involves a marked change in the relative proportion of the constituents. Persons with carious teeth differ from normal persons in that their normal resting saliva approximates in composition and neutralizing power to the composition of the activated saliva, in other words the salivary glands of such persons behave as though they were constantly receiving stimulus analogous to that constituted by the act of chewing a tasteless substance. Such a stimulus might conceivably be provided by carious teeth themselves, or on the other hand, both conditions may be attributable to a common underlying cause.

PART 2

RELATIONSHIP OF DENTAL CARIES AND THE COMPOSITION OF SALIVA TO DIETARY CONDITIONS AND THE LOCALITY AND NATURE OF STIMULI PROMOTING SECRETION

a. The relation of diet to the incidence of caries

The alteration in the salivary factor may be due to either a direct or an indirect cause. If the former, then the presence of dental caries in an otherwise healthy mouth would initiate the change. If the latter, then the change is either incidental or comprises a portion of a vicious circle.

Among the indirect factors which may initiate an acute disturbance may be mentioned diet, a lesion or an infection, a chronic peripheral nervous affection, a central nervous affection or lastly a defect in the processes of repair and growth correlated with a defect in salivary function.

Diet comprises per se two factors, namely composition and taste.

The former has been the subject of much discussion and research and the conclusions reached by the many authorities appear to be rather negative in character. Data hereinafter reported will deal more particularly with this phase.

It has been shown (6) that the salivary factor is constant in certain types of insanity to the same extent as in the normal individual and from this fact the deduction may be made that differences in neutralizing power are not related to the central nervous system.

In reviewing the literature concerned with the problems of dental caries and its possible relation to habits of cleanliness, climatic conditions, diet and general health of an individual or of a race, one notes a lack of uniformity in the recorded observations. This may be attributed to the fact that the many different writers were influenced in their judgment by different standards of observation, so that teeth which superficial examination would designate as non-carious might disclose, upon a more thorough examination, exactly the opposite condition.

Pickerill (1) states that certain food investigations point to the fact that the modern dietary of the civilized world differs from the diet of the uncivilized world in that the former is less hard but more tough and requires, therefore, more triturating but less crushing. From this conclusion the idea is advanced that, since different sets of muscles are used in triturating than in crushing, the over-development of these triturating muscles (buccinator and pterygoids) is responsible for both the abnormally shaped as well as the undeveloped dental arch. This, in turn, accounts for the increasing number of malposed teeth which accompany the under-developed arch. Malposed teeth are very susceptible to dental caries and therefore the increase of this disease among the modern civilized nations is correlated to our changed habits in masticating. Pickerill notes further that of the races of the world, the meat eaters, or at least those whose food is largely protein in character, were quite as susceptible to caries as those whose diet was mainly vegetarian. This is contrary to the views expressed by both Mummery and Patrick. The argument advanced by Pickerill is that the immune races, which include according to some authorities the Asiatics, Africans, Polynesians, Australians, et cetera, owe their comparative freedom from caries to the fact that their diets were both varied and sapid. Their universal use of masticatories resulted in the prevention of stagnation within the oral cavity (p. 314) a fact which other investigations appears to support.

Dr. R. Thurnwald, in speaking to the author of his anthropological researches in New Guinea, mentioned that the inhabitants in that section of the world seem to be comparatively free from dental caries. Their diet is mainly vegetable consisting of yams, sago, rice and sugar cane, etc.; meat is rather an accessory and, with the exception of the rather scarce mango, there are no acid fruits. The custom of chewing the betel nut plays, unintentionally, an important part, no doubt, in their oral prophylaxis. For at the age of puberty this custom, often connected with one of the initiatory ceremonies, is commenced and continued throughout the life of the individual.

Contrary to the usual belief, this betel nut habit does not blacken the teeth. For that purpose there is employed a mixture of cocoanut oil and soot which is vigorously rubbed on the teeth.

The betel nut is not used alone but is combined with seeds or leaves of a peppery nature together with pulverized lime from calcined shells. These three substances are taken into the mouth one after the other and are masticated between meals. The old men are edentulous and prepare a paste by mixing the material before chewing it. The natives expectorate profusely after the use of this mixture and the saliva is colored blood red. This coloration might be ascribed to the bleeding or to a compound formed by the action of the lime on the betel nut. The teeth are lost between the ages of forty and fifty and are exfoliated comparatively rapidly once the process has commenced. This exfoliation is accompanied by swollen and bleeding gums.

Calculus is deposited upon the teeth in almost unbelievable quantity, and it is often the case with the adults to see the size of the lower incisors increased by these concretions to 300 or 400 per cent.

Underwood (2) after examinations of skulls from different collections makes the following comment:

> In the hot belt of the earth including India, Africa and Southern China, bathing and washing are natural habits because of the heat; rinsing the mouth after meals and the use of sticks, toothpower, ashes and salt for cleansing the mouth is almost universal among the natives; while the food is largely rice and no alcohol is used. In all of them caries is so rare that to all intents and purposes the natives may be regarded as immune.

He states further that the people of the arctic regions whose personal habits, at least in regard to the care of the mouth, leave much to be desired, and whose diet is quite different, likewise enjoy immunity. He considers the Australian native equally immune. These observations lead him to conclude that the use of artificial foods and the re-

placing of breast feeding exerts a direct influence in the "weakening of the tooth defences." Just what constitutes "tooth defence" is not mentioned.

The effect of certain drugs upon the teeth has been dealt with by Austen (3). The systemic conditions which are supposed to favor the development of caries are anemia, dyspepsia, pregnancy, acute rheumatism, enteric and other continued fevers. Various salts and compounds of mercury, lead, bismuth, silver and copper were used in the experiments. Although it was found that the drug was partly excreted into the oral mucosa, yet it is rather an open question whether this excretion at one time may be so long continued as to accelerate or even cause any deleterious effect upon the erupted teeth. Another point however which may well be considered, is that the frequent drugging of growing children promotes a disturbance in the nutrition of the ameloblast and of the odontoblast thereby bringing about structural changes in the enamel and dentin respectively. Histological examinations conducted along this line of experimentation would undoubtedly throw light upon certain phases of present therapeutic methods.

In table 6 are presented certain abstracts and notations upon the teeth and diet of a few races from different parts of the world. Definite information on the subject appears to be rather scattered for in many instances an author may detail the foods at great length, the manner of cooking and habits of eating but will overlook entirely the conditions of the masticatory apparatus. In so far as the relative amounts of protein to carbohydrate in the diet are concerned, the data appear to confirm Pickerill's (1) conclusion in this regard, namely, that the protein eating races are as susceptible to dental caries as those whose food is mainly carbohydrate. On the other hand they appear to negative the popular impression that the teeth of primitive races are relatively immune to caries.

b. Relation of type and locality of stimulus to the neutralizing power of the secretion of saliva evoked by the stimulus

If the chronic disturbance of the neutralizing power be due to a chronic peripheral nervous affection or to a lesion or an infection remote from the salivary glands or teeth, such a disturbance would probably act through nervous reflexes. If the locality of the lesion is important, then by applying a definite stimulus to a circumscribed area in the oral mucosa such nervous irritation so produced might be expected to

TABLE 6

RACE	TEETH	DIET AND HABITS	AUTHORITY
Australian	"Toothache and indigestion common"	Large proteid diet. Flesh sometimes eaten raw. Only vegetable. "Nardu" grass, fern roots, yams	Eagle Bank and Crow, J. Mathieu. David Nutt, London
Australian	Teeth very tender	Chewing fiber from making of twine wears down teeth and makes them tender	The Australian Race, E. M. Curr. Tribunder & Co., London
Australian		Eat almost anything in the shape of animal food	Customs, Rites, etc., of the Aboriginal Tribe of the Gulf of Carpentaria, W. G. Stretton and E. C. Stirling. Trans. Roy. Soc. So. Australia, vol. 17, part 2, p. 240
Australian		Eat all the mammalian fauna	Report of the Horn Expedition, E. C. Stirling. Part IV, p. 51
Northern Australians		Natives eat anything that is edible	Native Tribes of the Northern Territory of Australia, Spencer. MacMillan & Co., 1914, New York
Brazilian	Unusually liable to decay	Milk, cheese, farina, sun dried meat. Food is bolted and all expectorate rather excessively	Highlands of Brazil, R. F. Burton. Tinsley Bros., London
Eesa or Bedoin branch of Somal	Teeth do not project, are poor and stained from surat tobacco	Mutton, milk, butter, rice, millet bread, dates	First Footsteps in East Africa, R. F. Burton. Dent & Co., London

Egyptians,* Ancient and Modern	Toothache common	Diet mainly vegetables, bread of millet or maize; milk, eggs, melons, beans, lupins. The cleaning of teeth a religious rite.	Account of Manners and Customs of Modern Egyptians, E. W. Lane. John Murray, London
Filipinos	Caries present in 20.90 per cent of cases examined	Diet includes great quantities of sugar	Ottofy Lancet, 176, 1909, 263
Friendly Isles	In general good teeth, but poorer than those of other nations	(See natives of Island of Otaheite)	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Gallois Tribe	(See Somal)	Gourds the only vegetable. Habitual drunkards. Habitually use the tooth stick	First Footsteps in East Africa, R. F. Burton. Dent, London
Igorots	Caries present in only 2.05 per cent of a large number of cases examined	Carnotes, a sweet potato, 30 per cent of diet. Rice, 30 per cent of diet. Other vegetables, maize, etc., 30 per cent of diet. Fish, meat and eggs, 10 per cent. Sugar is distasteful and is not used	Ottofy Lancet, 176, 1909. 253
Otaheite	White and regular	Mainly carbohydrate in character. Teeth cleansed six times a day	Captain Cook, Voyages of Discovery. Dent, London. Edited E. Phys.
Natives of Sarawak	"Toothache and decayed teeth are almost unknown"	Rice, yams, cucumbers, fowls, and fish	Natives of Sarawak and British North Borneo, H. L. Roth. Truslone & Hansone, London, p. 77.
Somal	Scattered teeth considered a sign of warm temperament	(See Eesa)	First Footsteps in E. Africa, R. F. Burton. Dent, London

* Note that Patrick quoted by Pickerell cites the Egyptians as one of the immune races, the ratio of caries in 3,306 teeth examined being only 3.418 per cent.

TABLE 6—Continued

RACE	TEETH	DIET AND HABITS	AUTHORITY
Somal	Teeth do not project, are poor and stained by tobacco	The teeth are cleaned by tooth stick. Flesh, holcus, few vegetables. Millet beer	First Footsteps in East Africa, R. F. Burton. Dent, London
Tasmanians	Among forty-two natives examined all had "very good teeth"	Refuse European food; eat rats, many vegetables of various kinds. Have enormous appetites. Meals are eaten with great greediness and great quantities are eaten at one time. Live chiefly on animal food	Aborigines of Tasmania, H. E. Roth. King & Son, Halifax, England, pp. 8 and 87
Tasmanians	Are reported by Cook, La Billardiere and Widowson as having good teeth		
Tasmanians	Teeth much worn; decay uncommon	Soundness of teeth due to chewing hard and tough material	Daily Life of the Tasmanians, Bonwick
Makololo	Beautiful teeth	General mixed diet, including:—porridge, milk, butter, corn, honey, beer, wild fruit, elephant meat	Narratives of an Expedition to the Zambesi, etc., David and Charles Livingstone. John Murray, London, 1865
Kebrabasa		Fowls, eggs, sugar cane, sweet potatoes, tomatoes, rice, maize; Kaffir corn	Ibid.
Manganja		Millet, maize, beans, nuts, yams, rice, pumpkins, cassava, sweet potatoes, etc.	Ibid.
Matumboka	"Teeth here are more solid and often wear down to the gums in old persons without decay. Cases of toothache not at all uncommon nevertheless"	Mixed diet	Ibid.

influence the neutralizing power. One of the easiest methods of stimulation is the use of an electric current which has been passed through an inductorium, and experiments along these lines were projected.

In this series of experiments it was desired to determine what comparative differences would result in titratable acidity and alkalinity by the use of the electric current at different parts of the oral mucosa. It has been demonstrated that the mechanical stimulus obtained by the chewing of paraffine excites a flow of saliva which is markedly different from that found normally in the mouth. With the employment of the electric current, obtained from an inductorium, a third sample was secured which differed in titration value from either the normal resting or paraffine saliva. The amount of current used and the locality at which it was applied did not appear to produce any marked deviation from the general result. Although the strength of current varied with different individuals, only that strength was used which at the end of two minutes produced a tingling sensation at the point of contact. Whenever this amount was appreciably increased it was found to be prejudicial to salivary activity, as an unnecessary nervous tension was thus produced.

The apparatus consisted of two Edison Lelande cells, type Z connected in series with an inductorium and a key. The electrodes consisted of two platinum points mounted on a vulcanite handle. The electrode was applied to the mucous membrane at some predetermined point and the saliva thus obtained titrated in the usual manner. The different localities at which the electrode was applied were, first, the opening of Stenson's duct opposite the upper second molar in the buccal mucosa; second, the openings of Wharton's ducts and the ducts of Bartholin on either side of the frenum linguae; third, on the dorsum of the tongue at the juncture of the posterior with the middle third near the apex of the V formed by the convergence of the two lines of the circumvallate papillae; fourth, at the gingivae. In applying the current at the bilateral structures one side was stimulated for two minutes and then the opposite side. No inflammation of the mucosa at the point of contact was developed at any time. The results of these experiments are reported in table 7. In the first column is noted the serial number of the patient, in each column "A" the alkalinity of 10 cc. of sample expressed in cubic centimeters of N/200 HCl.; in "B" the acidity of 10 cc. sample expressed in cubic centimeter of N/200 NaOH., and in "C" the total neutralizing power. The salivary factor appears whenever the paraffine saliva was taken; the distance of the

TABLE 7

"Electrical stimulus"

NUMBER	NORMAL RESTING SALIVA			PARAFFINE STIMULATED SALIVA			ELECTRICALLY STIMULATED SALIVA			FACTOR	COIL	DATE
	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.	HCl	NaOH	T. N. P.			
	A	B	C	A	B	C	A	B	C			
Stenson's duct												
	cc.	*cc.*		*cc.*	*cc.*		*cc.*	*cc.*			*cm.*	
E 1	14.05	5.25	19.30	25.50	2.35	27.85	4.80	16.50	21.30	69.30	27.50	December 28, 1915
E 2	15.30	5.80	21.10	34.40	3.25	37.65	18.20	5.50	23.70	56.50	25.50	January 5, 1916
E 2	4.10	15.30	19.40	24.50	3.40	27.90	9.45	11.00	20.45	69.50	27.00	December 28, 1915
E 4	6.60	13.75	20.35	24.15	4.75	28.90	7.55	17.75	25.30	70.40	30.00	January 7, 1916
E 5	17.70	24.75	42.45	32.75	3.35	36.10	17.80	8.50	26.30	117.60	27.00	January 10, 1916
E 6	19.50	6.50	26.00	48.30	−1.20	47.10	25.45	2.50	27.95	55.20	27.00	January 8, 1916
E 7	8.75	9.80	18.55	25.20	4.55	29.75	10.65	7.00	17.65	62.40	27.00	January 12, 1916
G 8	20.40	3.30	23.70	39.80	0.50	40.30	27.10	11.30	38.40	58.80	27.00	September 1, 1916
Wharton's and Bartholin's ducts												
E 1	9.00	9.50	18.50	17.75	5.50	23.25	10.75	5.30	16.05	79.60	30.00	January 5. 1916
E 2	16.95	1.30	18.25	34.70	−3.75	30.95	20.15	−1.50	18.65	58.90	32.00	January 12, 1916
E 4	17.80	4.60	22.40	19.40	8.25	27.65	15.90	7.10	23.00	81.00	29.50	January 8, 1916
F 1	16.25	6.95	23.20	29.50	2.70	32.20	19.05	6.25	25.30	72.09	30.00	January 15, 1916
F 2	21.10	3.40	24.50	34.20	1.90	36.10	19.30	3.10	22.40	67.90	29.75	January 16, 1916
F 3	20.10	3.10	23.20	24.15	2.75	26.90	15.00	2.30	17.30	86.20	29.50	January 17, 1916
F 3	16.10	6.25	22.35	22.00	1.85	23.85	14.40	2.50	16.90	93.70	29.50	January 18, 1916
F 5	17.70	4.50	22.20	37.40	2.50	39.90	14.25	3.95	18.20	55.70	29.00	January 18, 1916
G 7	22.85	2.50	25.35				19.90	2.70	22.60		29.50	February 22, 1916
G 8	15.15	6.50	21.65	44.85	1.50	46.35	27.55	11.00	38.55	46.71	27.80	July 22, 1916
G 8	26.05	7.75	34.80	44.55	3.10	47.65	26.40	11.80	38.20	74.70	26.00	August 16, 1916

Tongue

E 1	8.70	10.50	19.20	15.75	4.85	20.60	11.35	4.45	15.80	93.20	28.00	January 22, 1916
E 4	11.00	14.70	25.70				12.50	8.40	20.90		28.50	November 11, 1915

Gingivae

E 6	18.25	7.60	25.85				22.95	3.30	26.25		31.00	January 10, 1916
E 7	10.50	8.15	18.65				12.25	5.75	18.00		29.50	January 13, 1916
G 43	17.25	8.00	25.25	21.60	2.75	24.35	16.55	5.35	21.90	103.70	25.50	November 5, 1916
E 5	18.40	23.55	41.95				25.55	6.90	32.45		31.00	January 11, 1916

secondary coil from the primary shows the comparative strength of the current and is expressed in centimeters.

The data submitted demonstrate first, that the alkalinity of the electrically excited saliva is lower than that of the paraffine sample; second, that the acidity of the former is relatively higher than that of the latter; and third, that the neutralizing power of the saliva obtained by electric stimulus is lower in every instance than that collected by the paraffine method. This fact indicates that the use of the inductorium does not promote salivary activity to the same extent as the paraffine.

It was hoped that differential areas of irritation in the mouth could be demonstrated by means of this electrical stimulation for it was desired to determine whether one part of the oral mucosa is more sensitive to extraneous influences than another, so that a local irritation in one circumscribed locality would tend to produce a greater change in the salivary neutralizing power than in another locality. The experiments, however, so far reported, have failed to supply any conclusive evidence.

The repeated use of the inductorium on one individual produces a marked effect upon the relationship of the normal resting saliva to the activated paraffine saliva. This electric irritation alters the factor in a few days from one which is comparatively low to one with a much higher valuation. As examples of this condition may be cited subjects E1, E4 and G8. In the first instance the experiment was commenced on December 28, 1915. The salivary factor at this time was 69.3. Electrical stimulation was applied and after eight days a second test was made. The normal resting saliva and the paraffine saliva were first collected and the application of the current repeated. It was found that the factor rose steadily from 69.3 to 79.6 and finally to 93.2. Similarly with E4 the factor at the start was 69.5 but rose in eleven days to 81.0. For subject G8 the factor evaluated on July 23, 1916 was 46.7; on August 1 it had risen to 74.7. One month later it had returned to nearly the same ratio as at the start and duplicate samples on successive days gave a factor of 58.8. The results reported are based on duplicate analyses and on duplicate samples obtained on successive days.

The determination of the reaction of the taste impulses upon salivary secretion was attempted by comparing the results obtained with a normal resting sample with those secured by the use of different substances of marked taste. Howell (12) and other investigators describe the taste sense as being composed of four fundamental sensations, namely, bitter, sweet, acid and salty. Tastes other than these are combinations of any two or more of the primary sensations and produce, therefore, a

mixed stimulation of the sense organ. There was used for this experiment quinine on one day and sucrose on the second or third day following.

In securing the samples there was collected first a resting saliva and then the saliva secreted from the use of the taste stimulant. The results are reported in table 8. In the first column appears the subject number. Figures in columns A, B and C represent respectively the alkalinity, acidity and total neutralizing power of each sample. It may be stated in general that the action of sucrose, the sweet stimulant, tends to lower the total neutralizing power of the saliva. Quinine, the bitter stimulant, appears to produce the opposite condition yielding saliva resembling that secreted in response to the stimulus of chewing

TABLE 8

Comparison of effects of different types of stimulation upon salivary secretion

NUMBER	SALIVA NORMAL RESTING			SALIVA FROM QUININE STIMULUS			SALIVA FROM SUCROSE STIMULUS		
	A	B	C	A	B	C	A	B	C
F1	16.25	6.95	23.20	20.65	4.55	25.20	13.8[0]	10.70	24.50
F2	19.55	4.20	23.75	33.95	2.00	35.95			
F2	25.50	5.00	30.50	25.80	3.75	29.55	17.15	8.00	19.15
F2	21.15	7.50	28.65				18.45	5.50	23.95
F3	20.70	3.70	24.40				13.25	8.00	21.25
F3	18.40	5.40	23.80				9.95	8.25	18.20
F4	19.25	4.75	24.00				15.00	6.20	21.20
F5	19.10	5.75	24.85				10.65	8.00	18.65
G7	22.85	2.50	25.35				12.60	3.60	16.20
F1	18.05	4.70	22.75	19.60	13.40	33.00	13.85	7.00	20.85

paraffine. The well known work of Miller (13) demonstrates that fermenting carbohydrates such as would be found on the teeth tend to promote caries. From the above experiments it would be logical to infer that saliva favors the condition as well, since the neutralizing power of the sucrose-stimulated saliva approaches that of the normal resting saliva, and a factor evaluated on this basis would be of a magnitude corresponding to that which under the paraffine stimulus would indicate the presence of caries.

SUMMARY

Dialysis of saliva shows that the total neutralizing power is chiefly due to inorganic constituents.

The use of the Van Slyke apparatus in the determination of amino-nitrogen in saliva is a new application of this method. The results so

obtained show that there is a definite correlation between the concentration of inorganic constituents, the amino-nitrogen content, and the neutralizing power of saliva; namely, that a high neutralizing power is associated with a correspondingly high percentage of inorganic constituents and with a low percentage of protein.

Data are presented which confirm Pickerill's observations concerning the effect of different constituents of the diet upon dental caries.

The use of the electric current as a salivary stimulant excites a secretion markedly low in alkalinity and correspondingly high in acidity when compared to the saliva resulting from the paraffine stimulus. The neutralizing power of saliva secreted in response to electrical stimuli is less than that secreted in response to the chewing of paraffine.

Differential areas of stimulation in the oral mucosa cannot be demonstrated.

The comparison of analyses of saliva obtained by a sweet stimulus (sucrose) with that obtained by a bitter stimulus (quinine) proves that the former yields a saliva low in neutralizing power and that the latter produces the opposite result.

In conclusion I wish to express my deep appreciation to Dr. T. Brailsford Robertson for his interest evinced throughout the work as well as for the valuable suggestions offered. My thanks are also due to those students and clinic patients who have provided the necessary material for the experimental purposes.

BIBLIOGRAPHY

(1) Pickerill: The prevention of dental caries and oral sepsis, 2nd ed., Philadelphia, 1914.
(2) Underwood: Brit. Med. Journ., 1910, ii, 621.
(3) Austen: Brit. Med. Journ., 1910, ii, 772.
(4) Marshall: This Journal, 1915, xxxvi, 260.
(5) Shepard and Gies: Journ. Allied Dent. Soc., xi, 1916, 273.
(6) Marshall: This Journal, 1916, xl, 1.
(7) Gies: Journ. Allied Dent. Soc., xi, 1916, 488.
(8) Marshall: Dent. Cosmos, 1916, lviii, 1225.
(9) Marshall: Dent. Cosmos, 1917, lix, 33.
(10) Clausen: Journ. Biochem., 1914, xvii, 417.
(11) Porter: Quart. Journ. Exper. Physiol., 1910, iii, 375.
(12) Howell: Textbook of physiology, Philadelphia, 1915.
(13) Miller: Dent. Cosmos, 1902, xliv, 437.
(14) Hauk: Practical experimental physiology, 5th ed., Philadelphia, 1916.
(15) Davenport: Statistical methods, with special reference to biological variation, New York, 1904.

THE EPINEPHRIC CONTENT OF THE BLOOD IN CONDITIONS OF LOW BLOOD PRESSURE AND SHOCK[1]

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Very few investigations have been made to determine the existence of a possible relationship between the activity of the adrenal bodies and shock although the reactions of the vascular system to injections of epinephrin and its condition during shock indicate that some such relationship may exist.

The results thus far reported apparently indicate that during shock the chromaffin material disappears from the adrenal bodies, and the conclusion has been drawn that low blood pressure and shock are accompanied by a lack of epinephrin in the blood. As logical as this conclusion appears to be, it does not seem to harmonize with a number of observed phenomena. Furthermore an examination of the published reports of these researches indicates a possible error in arriving at this conclusion.

Consequently the present investigation was undertaken with the view to determine by an examination of the amount of epinephrin in the blood of the adrenal vein, the activity of the adrenal bodies during the following conditions, productive of shock.

a. Handling of the intestine.

b. Low blood pressure from hemorrhage.

c. Occlusion of the inferior vena cava.

The only record of experimental evidence as to the quantity of epinephrin in the blood during conditions associated with shock is unaccompanied by details.

METHODS

All experiments were performed on dogs. Examination of the literature indicated that the use of the intestinal strip of the rabbit

[1] Preliminary report of this work was published in the Proceedings of the Society for Experimental Biology and Medicine, xiii, No. 5, 1916.

recommended by Hoskins (9) because of its specificity and degree of reaction, furnished the best means for the detection of variations in the amount of epinephrin in the blood.

It was necessary to provide an apparatus that permitted immersion of the intestinal strip in the medium to be tested at body temperature. Provision also had to be made for the rapid replacement of one medium with another and for passing through the fluid a regular supply of oxygen. Intestinal strips were obtained in the usual manner from a urethanized rabbit.

The abdominal wall was opened, and pieces of small intestine 3 cm. to 4 cm. in length, removed, if possible, from a region devoid of intestinal contents. Each piece (apparatus was made in duplicate), to be used immediately, was arranged in the glass tube used for holding the medium tested and attached by pin hooks to a thread connected with a recording needle. Other pieces to be used as a reserve were placed in a beaker of Ringer's solution through which was passed a continuous stream of oxygen and kept on a water bath at a temperature of 37°C. After considerable experimentation, it was found that the intestinal strip contracted most satisfactorily in a Ringer's solution containing NaCL 0.85 per cent, $CaCl_2$ (crystals dried) 0.026 per cent and KCl 0.03 per cent. The strips usually began at once to contract rhythmically at the rate of about 13 contractions per minute. Strips of intestine frequently continued to react for four or five hours with only slight diminution in amplitude and rate. During this time, however, the Ringer's solution was replaced from time to time and constantly a stream of oxygen bubbled through the liquid.

Intestinal strips from very young rabbits gave poor results and occasionally strips from adult rabbits contracted poorly.

The rhythmic contractions of the intestine were diminished in amplitude and the tone of the strip decreased by replacing the normal Ringer's solution with Ringer's holding in solution, small quantities of adrenalin (adrenalin put up by Parke, Davis and Company was used). A very definite reaction was produced in dilutions as great as 1–500,000,000. Dilution of 1–1,000,000 brought about complete inhibition of movement and great loss of tone. It was noticed that the strip after a time became less sensitive to the adrenalin so that a larger quantity was required to completely stop the contractions. At the same time the ability of the strip to detect minute quantities was lessened.

The rhythmic contractions of the strips were tested in rabbit's blood

and then in dog's blood. The strip immersed in blood contracted in the same manner as in Ringer's solution, except that usually the tone increased very markedly when the blood first replaced the Ringer's. Sometimes the first contractions in blood were very irregular especially in amplitude, but in a few minutes they became quite uniform. It was found that the most satisfactory results were obtained when the blood was diluted one-half with Ringer's solution. This dilution was used in all of the experiments.

The reaction of the intestinal strip to this diluted dog's blood containing small quantities of adrenalin was tested with results similar to those of Ringer's solution and adrenalin.

The general procedure in the experiments was as follows:

A physiological dose of morphine was injected into the dog before ether anesthetization. During the course of the experiment, no more ether was administered. As soon as the dog failed to display the ordinary reflexes, the right jugular vein was dissected free, a cannula inserted and from this about 50 cc. of blood drawn to be used as a control. All blood was defibrinated, as soon as drawn, and placed on ice. The left carotid artery was laid bare, and a cannula was inserted and connected by a rubber tube to a mercury manometer for recording blood pressure. The abdomen was then opened along the linea alba. By means of towels, wrung out in water at 37°C., the intestines were pushed to the left of the mid-line to expose the abdominal vena cava. The vena cava, right renal vein and inferior mesenteric vein were dissected free from surrounding tissue. The renal vein branches of the vena cava and a branch of the inferior mesenteric were ligatured, the latter as far distally as possible, usually an inch or an inch and a half from its entrance into the vena cava. A cannula was inserted into the inferior mesenteric proximal to the ligature, and pushed in until its end was well within the vena cava, and opposite to the opening of the adrenal vein. It was necessary to obtain blood, both before and during a condition of shock from the adrenal vein unmixed with vena cava blood, yet in the interval between the two drawings, the general circulation and the pouring of adrenal blood into the vena cava must not be interfered with. At first for the purpose of preventing the mixture of vena cava with adrenal blood during the period of drawing blood, loose ligatures were laid around the vena cava, proximal and distal to the entrance of the adrenal vein into the vena cava. At the time of drawing the blood the ligatures were firmly pulled to completely occlude the vena cava at these two points, making a closed chamber with the adrenal vein

as the only opening into it. It was found that the use of these ligatures was not satisfactory, as in the process of drawing the blood, it was difficult to make certain that the vena cava was completely occluded during the entire time; and the traction on the vena cava had a tendency to pull the adrenal vein from its normal position, thus interfering with the regular output of blood from it. After experimenting with various kinds of clamps, some with curved jaws protected by rubber and fitted with handles, were obtained, which gave very satisfactory results.

The first blood drawn, equal approximately to that enclosed in the section of vena cava, was rejected; the following blood was carefully measured as to rate of flow in order to eliminate the possibility that results obtained might be due to a greater concentration of epinephrin because of the less rapid flow of blood through the organ although its activity might not have been increased. The blood was defibrinated. After sufficient blood had been drawn, the clamps on the vena cava were released and a small amount of citrate put into the cannula to prevent coagulation. The cannula was then clamped off and the abdominal cavity closed as completely as possible, until after shock had been brought about by one of the methods employed.

At the time of drawing blood, during or after the development of shock, the same procedure was followed. At this time the blood pressure was usually low and trouble was frequently experienced, at first, with coagulation interfering with the flow of the blood, but later, with more care in cleansing the cannula, this was obviated.

After the termination of the experiment, a post mortem examination was made to be certain that no unligatured veins were connected with the portion of the vena cava between the clamps.

The following designations have been given to the various samples of blood:

Control blood. Blood taken from jugular vein before operation.

Blood No. 1. Blood taken from adrenal vein before shock has been induced.

Blood No. 2. Blood taken from adrenal vein after development of shock.

Blood Nos. 3, 4, 5. Blood taken at successive periods during and after development of shock.

The intestinal strip was found to contract regularly in jugular blood, this blood was replaced by blood No. 1 (blood taken from adrenal vein before shock). After a short time the strip was placed in blood taken during or after development of shock (blood Nos. 2, 3, 4 or 5).

As will be seen by examination of tracings, the results were checked by varying the order of replacements. To determine the quantitative relation of epinephrin in the samples tested, two methods were employed. In the one the tracings obtained were compared with those obtained by the addition of known quantities of adrenalin. In the second method the blood giving the reaction of epinephrin was diluted with control blood until the reaction of this blood was similar to that of blood No. 1.

Two classes of control experiments were conducted:

1. Experiments in which the manipulative processes and time elements were the same as in the regular experiments, but a condition of shock was not induced.

2. Experiments in which the adrenal bodies were ligatured in such a way that while the blood from the lumbar branch of the adrenal vein was permitted to enter the vena cava, no material could pass from the adrenal gland into the circulation.

The means of inducing low blood pressure and shock were as follows: (1) manipulation of the intestine, (2) hemorrhage, (3) occlusion of vena cava.

1. Manipulation of the intestine. After drawing blood from the adrenal vein, a considerable portion of the intestine was drawn out and handled until a condition of shock was brought about in the animal. This occurred in from one to two hours. The blood pressure at the end of the period of handling varied from 40 mm. to 60 mm. Hg.

2. Hemorrhage. Blood was allowed to flow slowly from either the vena cava, femoral or jugular vein. Usually from 50 cc. to 100 cc. of blood were taken at intervals of ten or fifteen minutes. After clamping off the cannula there was a tendency for an increase in blood pressure to occur. As long as this tendency manifested itself, blood at intervals was taken. After the blood pressure had remained stationary at 40 to 50 mm. Hg for from thirty to sixty minutes, blood No. 2 was drawn.

3. Occlusion of vena cava. Interrupted intratracheal insufflation was given.

The skin and tissue were cleaned away from one of the ribs. By means of a periosteal elevator, the rib was separated from its periosteum and a piece about 2 inches in length cut away with bone forceps. The periosteum and pleura were carefully cut through and by means of a curved aneurysm needle, a thread was passed around the thoracic inferior vena cava. The two ends were brought through the incision so that the vena cava might be occluded to any desired extent

by pulling upon the two ends of the thread. The incision was sewed up; the respiration machine was disconnected and the animal resumed its normal method of respiration.

As will be seen from descriptions of the experiments the pressure was reduced considerably and held at that point for some time. In many cases it was still further reduced, and the drawing of the blood did not occur until a tendency to fall became evident in the blood pressure.

DESCRIPTION OF EXPERIMENTS

Handling Experiments

Dog 2. Weight, 7 kgm.

Pressure before operation, 140 to 150 mm. Hg

11.00 a.m. Rate of blood flow from adrenalin vein, 6 cc. per thirty seconds

No. 1 blood

11.00 a.m. Pressure at beginning of handling, 120 mm. Hg
1.00 p.m. Pressure after two hours handling, 120 mm. Hg
1.15 p.m. Pressure after two hours fifteen minutes handling, 120 mm. Hg
1.15 p.m. Rate of blood flow after two hours, fifteen minutes handling, 6 cc. per thirty seconds

No. 2 blood

3.00 p.m. Pressure after four hours handling, 70 mm. Hg
Blood in cannula clotted. No blood could be drawn.

Note that in this experiment there was no fall of pressure after over two hours handling, although the dog exhibited the usual symptoms of shock, with exception of low blood pressure. The blood taken after handling of intestine for two hours and fifteen miuntes exhibited relatively large quantities of epinephrin. Even after dilution of thirty-two times with jugular blood No. 2 blood showed greater quantities of epinephrin than blood No. 1.

Dog 5. Weight, 11 kgm.

9.50 a.m. Blood pressure before operation, 120 to 130 mm. Hg
10.15 a.m. Rate of flow from adrenal vein, 5 cc. per thirty seconds or 1 cc. per sixty seconds.

No. 1

10.15 a.m. Blood pressure at beginning of handling, 110 to 120 mm. Hg
10.45 a.m. Blood pressure 110 to 120 mm. Hg
11.00 a.m. Blood pressure 120 to 130 mm. Hg
11.15 a.m. Blood pressure 120 to 130 mm. Hg
11.30 a.m. Blood pressure 120 to 130 mm. Hg
12.10 p.m. Blood pressure 80 to 90 mm. Hg
12.15 p.m. Blood pressure after handling of intestine two hours 60 to 70 mm. Hg

12.15 p.m. Rate of flow from adrenal vein, No. 2 blood, 4 cc. per sixty seconds or 1 cc. per fifteen seconds

After diluting blood No. 2 two and one-half times to compensate for the slower flow (on the supposition that the adrenals manufacture the same amount of epinephrin regardless of the amount of blood supplied to them), it was necessary to further dilute the blood two and one-half times with jugular blood before a tracing was given which resembled that given by blood No. 1.

Fig. 1. Dog 2. Handling intestine. Blood after shock (2) diluted thirty-two times with jugular blood still showing presence of epinephrin.

Dog 6. Weight, 4.5 kgm.

9.50 a.m. Blood pressure before operation, 110 to 120 mm. Hg
10.15 a.m. Rate of blood flow from adrenal vein, No. 1 blood, 4.5 cc. per sixty seconds
10.20 a.m. Blood pressure at beginning of handling, 85 to 95 mm. Hg
10.55 a.m. Blood pressure, 65 to 75 mm. Hg

11.05 a.m. Blood pressure, 50 to 60 mm. Hg
11.20 a.m. Blood pressure, 30 to 40 mm. Hg
11.20 a.m. Rate of flow from adrenal vein, 2 cc. per sixty seconds. No. 2 blood.

After dilution of No. 2 blood with jugular blood to compensate for difference of flow, the tracing made by No. 2 blood showed approximately the same difference from the tracing made by No. 1 blood as the difference between the tracings of normal jugular blood and jugular blood to which had been added adrenalin sufficient to make a content of 1–10,000,000.

HEMORRHAGE EXPERIMENTS

Dog 8. Weight, 8 kgm.

9.50 a.m. Blood pressure before operation, 110 to 120 mm. Hg
9.50 a.m. Rate of flow of blood from adrenals 3.5 cc. per sixty seconds
10.15 a.m. Blood drawn, pressure fell to 40 mm. Hg
10.40 a.m. Blood pressure rose to 100 mm. Hg
10.52 a.m. / 10.57 a.m. Blood drawn, pressure fell to 40 mm. Hg
11.10 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.12 a.m. / 11.18 a.m. Blood drawn, pressure fell to 60 mm. Hg
11.24 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.25 a.m. / 11.27 a.m. Blood drawn, pressure fell to 40 mm. Hg
11.35 a.m. Blood pressure rose to 60 to 70 mm. Hg
12.20 p.m. Blood pressure began to fall 40 mm. Hg
2 cc. Blood drawn from adrenal vein, pressure fell to 20 to 30 mm. Hg
50 cc. Normal salt injected into vena cava blood pressure rose to 40 mm. Hg
2 cc. Blood drawn from adrenal vein. Rate of flow from adrenal vein 2 cc. per sixty seconds

After dilution with jugular blood to compensate for difference in rate of flow No. 2 blood showed indications of containing considerably more epinephrin than No. 1, but the difference in this experiment was not so marked as in some other experiments. This difference amounted to an addition of sufficient epinephrin to make a dilution of 1–50,000,000.

Dog 15. Weight, 11 kgm.

Blood pressure before operation, 120 to 130 mm. Hg
11.00 a.m. Blood from adrenal vein, rate of flow, No. 1, 9 cc. per thirty seconds
11.00 a.m. Blood pressure, 110 to 120 mm. Hg
11.15 a.m. Blood drawn, blood pressure fell to 80 to 90 mm. Hg
11.25 a.m. Blood pressure rose to 100 to 110 mm. Hg
11.30 a.m. Blood drawn, blood pressure fell to 50 to 60 mm. Hg
11.35 a.m. Blood pressure rose to 80 to 90 mm. Hg
11.36 a.m. Blood drawn, blood pressure fell to 50 to 60 mm. Hg

11.50 a.m. Blood pressure rose to 60 to 70 mm. Hg
11.52 a.m. Blood drawn, pressure fell to 35 to 45 mm. Hg
12.10 p.m. Blood from adrenal vein, rate of flow No. 2, 3 cc. per thirty seconds
12.10 p.m. Blood pressure 20 cc. normal NaCl injected, 20 to 30 mm. Hg
12.30 p.m. Blood pressure fell to 15 to 20 mm. Hg
Blood from adrenal vein, rate of flow No. 3, 2 cc. per thirty seconds
Pressure fell while drawing blood.
12.40 p.m. Pressure 0. Animal died.

Examinations of tracings show that even after dilution seventeen times with jugular blood, blood No. 2 contained more epinephrin than blood No. 1.

Fig. 2. Dog 15. Bleeding

Dog 16. Weight, 17 kgm.

10.00 a.m. Blood pressure before operation, 135 to 140 mm. Hg
10.30 a.m. Rate of flow from adrenal vein, No. 1, 8 cc. per thirty seconds
10.30 a.m. Blood pressure, 110 to 120 mm. Hg
10.30 a.m. } Blood drawn, (40 cc.), pressure remained at 110 to 120 mm. Hg
10.35 a.m. }
10.40 a.m. Blood drawn, (50 cc.), pressure remained at 110 to 120 mm. Hg
10.45 a.m. Blood drawn, (50 cc.), pressure remained at 110 to 120 mm. Hg
10.50 a.m. Blood drawn, (70 cc.), pressure remained at 110 to 120 mm. Hg
11.00 a.m. } Blood drawn, (100 cc.), pressure 100 mm. Hg
11.05 a.m. }

11.10 a.m. Blood drawn, (50 cc.), pressure 100 mm. Hg
11.25 a.m. Blood drawn, (50 cc.), pressure rose to 115 mm. Hg
11.35 a.m. Blood drawn, (100 cc.), pressure 110 mm. Hg
11.40 a.m. Blood drawn, (70 cc.), pressure fell to 95 mm. Hg
12.00 m. Blood drawn, (100 cc.), pressure fell to 60 mm. Hg
12.05 p.m. Blood drawn, (50 cc.), pressure fell to 50 mm. Hg
12.25 p.m. Blood drawn, rose to 60 mm. Hg
12.35 p.m. Blood drawn, fell to 50 mm. Hg
12.40 p.m. Blood drawn, fell to 30 mm. Hg
1.00 p.m. Rate of flow of blood from adrenal vein, No. 2, 4 cc. per thirty seconds
Pressure fell rapidly, animal died.

It will be noticed that pressure failed to fall for a long period, and that when it began to fall it did so very rapidly.

From examination of tracings, it was seen that both No. 1 and No. 2 blood contained large quantities of epinephrin as compared with jugular blood, but no striking difference in amount in blood No. 1 and blood No. 2. Blood No. 2 contained additional epinephrin equal to an amount sufficient to make a dilution of 1–25,000,000.

VENA CAVA OCCLUSION EXPERIMENTS

Dog 12. Weight, 17 kgm.

Blood pressure before operation, 120 to 125 mm. Hg
11.40 a.m. Rate of flow of blood from adrenal vein, 12 cc. per thirty seconds

No. 1 blood

11.40 a.m. Blood pressure, 120 mm. Hg
11.45 a.m. Vena cava occluded, blood pressure, 60 mm. Hg

Kept at this pressure until 1.15

1.15 p.m. pressure began to fall rapidly falling to almost 0 mm. Hg

Vena cava was clamped above and below adrenal vein and blood from enclosed section removed with pipette. This blood was evidently blood returning from posterior part of body, by way of vena cava, inferior mesenteric and adrenal veins, practically general venous blood. It will be seen by referring to tracings that this blood contains a very much greater amount of epinephrin, equal to an amount sufficient to make a dilution of 1–10,000,000. If no increased output of epinephrin occurred, then this No. 2 blood should show less epinephrin than No. 1 blood, because of the great admixture of general venous blood.

Dog 14. Weight, 31 kgm.

Blood pressure before operation, 140 mm. Hg
Rate of flow of blood from adrenal vein, No. 1, 10 cc. per thirty seconds
12.00 m. Blood pressure, 120 mm. Hg
12.10 p.m. Blood pressure reduced by occlusion, 50 mm. Hg
12.55 p.m. Blood from adrenal vein, rate of flow No. 2, 7 cc. per thirty seconds

Blood pressure kept at 50 mm. until 1.30 p.m.

1.30 p.m. Blood from adrenal vein, rate of flow No. 3, 7 cc. per thirty seconds, 50 mm. Hg

2.05 p.m. Blood from adrenal vein, rate of flow No. 4, 7 cc. per thirty seconds

2.18 p.m. Blood pressure lowered to 35 mm. Hg

Blood pressure kept between 30 and 35 mm. for twenty minutes

2.38 p.m. Blood pressure showed indication of falling.

2.40 p.m. Blood from adrenal vein, rate of flow No. 5, 5 cc. per thirty seconds

2.50 p.m. After rapid fall of pressure, animal died.

Fig. 3. Dog 12. Occlusion of vena cava

Examination of tracings show somewhat increasing amounts of epinephrin in the blood with the continuance of the low blood pressure of 50 mm. Hg. The greatest differenc is evident in blood which had been kept at a pressure of 30 to 35 mm. Hg, after the one hundred and five minutes at pressure of 50 mm. Hg. Evidently a very greatly increased amount of epinephrin has been added to blood during the period of this very low pressure.

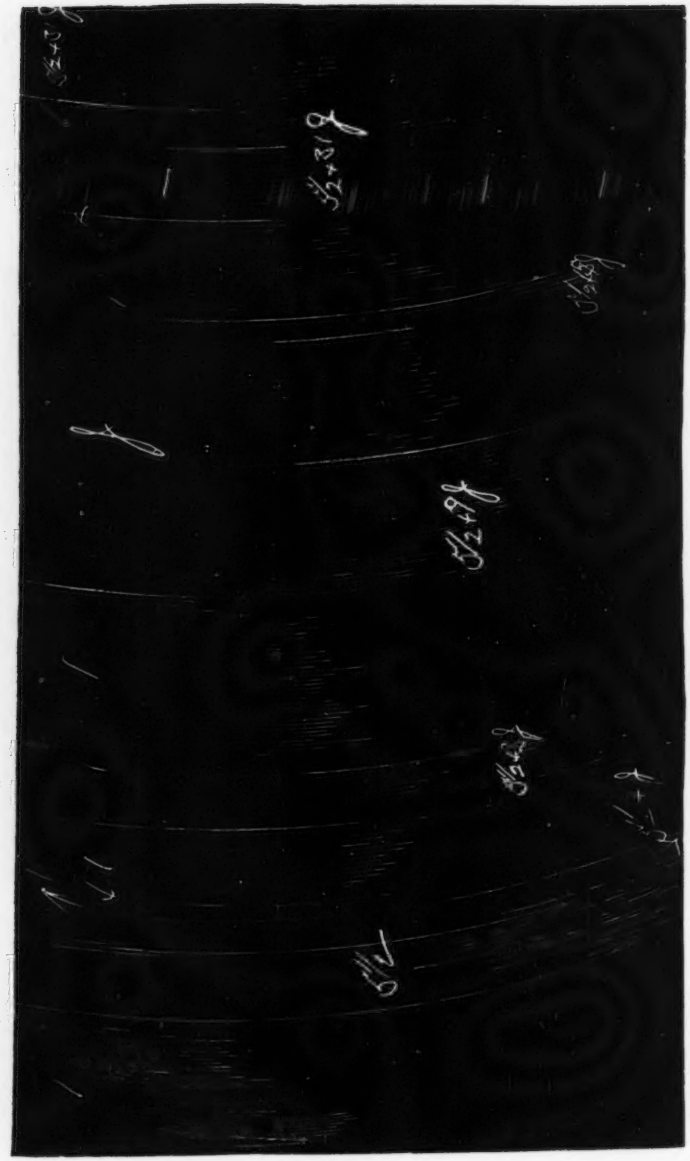

Fig. 4. Dog 14. Occlusion of vena cava

Dog 17. Weight, 17.5 kgm.

Blood pressure before operation, 140 mm. Hg

11.00 a.m. Blood from adrenal, rate of flow No. 1, 8 cc. per thirty seconds

11.05 a.m. Blood pressure, 130 mm. Hg

Blood pressure held at 60 mm. until 12.05.

12.05 p.m. Blood from adrenal vein, rate of flow No. 2, 9 cc. per thirty seconds

12.10 p.m. Blood pressure reduced to 40 mm. Hg

Blood pressure held at 40 mm. Hg until 12.55.

12.55 p.m. Blood from adrenal vein, rate of flow No. 3, 10 cc. per thirty seconds

1.00 p.m. Blood pressure reduced to 30 mm. Hg

Blood pressure held at 30 mm. Hg until 1.10 p.m.

1.10 p.m. Blood from adrenal vein, rate of flow No. 4, 8 cc. per thirty seconds

1.15 p.m. Blood pressure held at 30 mm. until 1.25.

1.25 p.m. Blood pressure began to fall, pressure failed to react on release of ligature around vena cava, although up to this time the pressure rose somewhat on release of tension of ligature.

1.26 p.m. A few cubic centimeters of blood were obtained from adrenal vein. No. 1.

Fig. 4a. Dog 14. Occlusion of vena cava

Examination of tracings shows that blood No. 2 and blood No. 3 contain no greater amount of epinephrin than blood No. 1. Blood No. 2 was drawn after pressure had been kept at 40 mm. Hg for one hour; blood No. 2 was drawn after pressure had been kept down to 30 mm. Hg, for an additional period of fifty minutes. Blood No. 4 and especially blood No. 5 show the presence of larger additional amounts of epinephrin. Blood No. 4 was taken after pressure had been kept to 30 mm. for an additional fifteen minutes and at a time when the power of reaction of the blood vessel had disappeared. Evidently an increased amount of epinephrin was poured into the blood as the period of low pressure became prolonged and at a time when the blood pressure mechanism was becoming ineffective.

CONTROL EXPERIMENTS

Dog 7. Weight, 15 kgm.

9.40 a.m. Pressure before operation, 120 to 130 mm. Hg
10.20 a.m. Blood from adrenal vein, rate 25 cc. per thirty seconds of flow No. 1
10.20 a.m. Blood pressure, 100 mm. Hg
11.20 a.m. Blood pressure reduced by bleeding to 40 to 50 mm. Hg
11.20 a.m. Blood from adrenal vein, rate of flow No. 2, 12 1.2 cc. per second
Blood No. 2 showed no greater amount of epinephrin than blood No. 1.

This experiment shows several things; first, that the manipulation, incident to the operation, and remaining under ether, for the length of time of an experiment, does not cause an increased amount of epinephrin in the blood; second, that the low pressure from bleeding must be maintained for a considerable time before an increased amount of epinephrin in the blood can be observed.

Dog 18. Weight, 1.5 kgm.

Blood pressure before operation, 120 mm. Hg

Abdominal cavity was opened and adrenals were tied off so that blood flow was interfered with as little as possible.

This was accomplished for the left suprarenal but ligature of right apparently interfered somewhat with blood flow. The manipulation caused a fall of blood pressure to 80 mm. Hg.

Blood was taken from vena cava through inferior mesenteric vein, blood No. 1.

By occlusion of vena cava pressure was reduced to 50 mm. Hg and kept at that pressure for one hour and thirty minutes. The blood pressure was then reduced to 30 mm. and kept at that point for thirty minutes, when the blood pressure began to fall. Blood was then drawn as before, from the vena cava, through the inferior mesenteric vein.

Examination of tracings shows that blood No. 2 had no greater tendency to cause relaxation of strip of rabbit intestine than blood No. 1. On the contrary just the reverse was true as might be expected if the change in the intestinal strip was due to the presence of epinephrin in the blood. The tracings indicate that as soon as blood No. 1 was replaced by blood No. 2, the tone of the strip increased.

This experiment indicates that no other substance than epinephrin poured into the blood, e.g., excretion of pituitary body, produces the characteristic reaction of the intestinal strip.

Dog 19. Weight, 10 kgm.

Blood pressure before operation, 120 mm. Hg
Adrenal glands ligatured in the same manner as in the preceding experiment
During the operative processes, blood pressure fell to 90 mm. Hg
12.00 m. Blood drawn from vena cava through inferior mesenteric vein
Pressure reduced to 50 mm. Hg. By tension on thoracic inferior vena cava and kept at that point until 1 p.m.

Fig. 5. Dog 17. Occlusion of vena cava. Pressure reduced to 30 mm. No. 3: Pressure reduced to 40 mm. after one hour five minutes reaction to 80 mm. No. 4: Pressure reduced to 30 mm. after one hour fifty-five minutes reaction to 80 mm. No. 5: Pressure reduced to 30 mm. after two hours twenty minutes; no reaction.

1.00 p.m. Blood pressure reduced to 40 mm. Hg
1.15 p.m. Blood pressure reduced to 30 mm. Hg
1.30 p.m. Blood drawn from vena cava through inferior mesenteric vein, No. 2
1.30 p.m. Blood pressure reduced to 20 mm. Hg
1.40 p.m. Blood pressure falling, blood drawn from vena cava through inferior mesenteric, No. 3

Examination of tracings shows that neither No. 2 blood nor No. 3 blood had any effect in causing a loss of tone or relaxation of the intestinal strip of rabbit.

It is evident therefore, from this experiment and the previous one that the relaxation of the intestinal strip in a condition of prolonged low blood pressure condition is due to epinephrin from the adrenal glands.

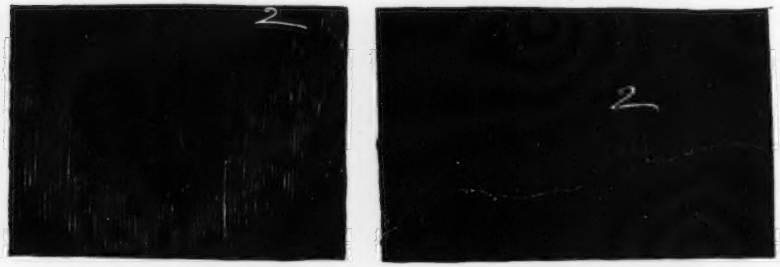

Fig. 6. Dog 18. Low blood pressure by occlusion of vena cava, suprarenals ligatured.

SUMMARY OF RESULTS OF EXPERIMENTS

In all three types of experiments, the epinephric content of the adrenal blood was increased provided that the pressure was sufficiently low and the condition of low pressure maintained for a sufficient length of time. Since the blood was diluted with control blood to compensate for the difference in the rate of flow through the adrenal organ, an increased activity of these organs was indicated.

It is a question whether this dilution of the blood to compensate for the difference in rate of flow through the adrenal bodies is legitimate. The dilution is made on the supposition that possibly the absolute output of the glands is not affected by the blood supply; that when the rate of flow of blood through the gland is one-half as great, this blood will contain twice as much epinephrin per cubic centimeter as blood flowing through the gland at the normal rate. As such a difference is not probable, the blood taken after shock in these experiments has evidently been over diluted and the results are more positive than they appear to be.

It is of interest to note in this respect that in the experiments with animals 2 (fig. 1) and 7, the rate of flow of the blood taken after shock was identical with that of the blood taken before shock. In both these cases, however, a relatively greatly increased amount of epinephrin was found in the blood drawn from the adrenal vein, after shock had developed.

In one case it was necessary to dilute the experimental adrenal blood with thirty-two times (fig. 1) its volume of jugular blood and in another thirty-one times (fig. 4) before a tracing could be obtained similar to that of adrenal blood drawn before low blood pressure was induced. In other cases the reaction was similar to the reaction given by control blood to which had been added adrenalin sufficient to make a 1–10,000,000 dilution, animal 12, which, in comparison with the normal amount of epinephrin in the blood, is a much more concentrated solution.

While the accurate determination of the actual amount of epinephrin in the blood may be difficult, yet there has never been any doubt as to the general relative amounts in samples of blood taken during one experiment.

In experiments in which the degree of low blood pressure could be most accurately controlled samples taken at intervals showed that the marked increase of epinephric content of the blood occurred only after a considerable duration of a condition of low blood pressure, varying from one to two hours (figs. 4 and 5). In these experiments, the later samples indicated an increasing amount of epinephrin.

None of the experiments showed any indication of a lessened amount of epinephrin in the blood, even after the most prolonged period of low blood pressure and although the animal might be at the point of death.

The handling experiments showed that in cases of shock accompanied by high blood pressure, the blood contains relatively a large amount of epinephrin. In some instances negative results were obtained unless the pressure was permitted to fall below 50 mm. to 60 mm. Hg. These negative experiments served as controls, indicating that the anesthetization and general operative procedure did not bring about the results obtained. To be certain that the results were not due to the presence in the blood of the secretion of some other organ, for example the pituitary body, the adrenal bodies were eliminated by ligatures. The results in these cases, however vigorous the experiment, were negative (fig. 6).

Table of experiments

NUMBER OF DOG	MEANS OF INDUCING SHOCK	BLOOD PRESSURE BEFORE SHOCK	TIME BETWEEN TAKING BLOODS NUMBER 1 AND NUMBER 2	PERIOD OF VARIOUS STAGES OF BLOOD PRESSURE	RESULTS	
					By dilution	Increase of epinephrin by comparison with known quantities of adrenalin
		mm. Hg				
2	Handling of intestine	120	2 hr. 15 min.	120 mm. Hg–2 hr. 15 min.	32×+	
5	Handling of intestine	115	2 hr.	115 mm. Hg–45 min. 125 mm. Hg–30 min. 85 mm. Hg–40 min. 65 mm. Hg– 5 min.	2½×	
6	Handling of intestine	90	1 hr.	90 mm. Hg–35 min. 70 mm. Hg–10 min. 55 mm. Hg–15 min. 35 mm. Hg– 1 min.		Additional 1–10,000,000
8	Hemorrhage	115	2 hr. 30 min.	115 mm. Hg–65 mm. Hg–1 hr. 45 min. 65 mm. Hg–45 min. Pressure fell rapidly to 40 mm. Hg.		Additional 1–50,000,000
9	Hemorrhage	100	1 hr. 50 min.	100 mm. Hg–30 mm. Hg–1 hr. 30 mm. Hg–50 min.		Additional 1–50,000,000
15	Hemorrhage	125	1 hr. 10 min.	125 mm. Hg–65 mm. Hg–50 min. 65 mm. Hg–20 min.	17×+	

16	Hemorrhage	115	2 hr. 30 min.	115–110 mm. Hg–1 hr. 10 min. 95 mm. Hg–20 min. 60 mm. Hg–40 min. 30 mm. Hg–20 min.		Additional 1–25,000,000
12	Occlusion of vena cava	120	1 hr. 35 min.	60 mm. Hg–1 hr. 30 min. Pressure then fell rapidly		Additional 1–10,000,000
14	Occlusion of vena cava	120	55 min.	50 mm. Hg–45 min. Blood Number 2	10×	
				50 mm. Hg–80 min. Blood Number 3	15×	
				50 mm. Hg–115 min. Blood Number 4	20×	
				50 mm. Hg–115 min. 30–35 mm. Hg–20 min. Blood Number 5	31×+	
17	Occlusion of vena cava	130	60 min.	60 mm. Hg–60 min. Blood Number 2	1X	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. Blood Number 3	1×	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. 30 mm. Hg–15 min. Blood Number 4	2×	
				60 mm. Hg–60 min. 40 mm. Hg–50 min. 30 mm. Hg–30 min. Pressure began to fall rapidly Blood Number 5	11×+	

Check experiments

NUMBER OF DOG	MEANS OF INDUCING SHOCK	BLOOD PRESSURE BEFORE SHOCK	TIME BETWEEN TAKING BLOODS NUMBER 1 AND NUMBER 2	PERIOD OF VARIOUS STAGES OF BLOOD PRESSURE	RESULTS	
					By dilution	Increase of epinephrin by comparison with known quantities of adrenalin
		mm. Hg				
7	Hemorrhage	100	60 min.	100–50 mm. Hg–60 min.	1×	
18	Occlusion of vena cava	120	90 min.	50 mm. Hg–90 min. 30 mm. Hg–30 min.	1×−	Adrenals ligatured
19	Occlusion of vena cava	120	90 min.	50 mm. Hg–60 min. 40 mm. Hg–15 min. 30 mm. Hg–15 min. Blood Number 1	1×	Adrenals ligatured
				50 mm. Hg–60 min. 40 mm. Hg–15 min. 30 mm. Hg–15 min. 20 mm. Hg–15 min. Blood Number 3	1×	Adrenals ligatured

× = the amount of epinephrin in Blood Number 1.

DISCUSSION OF RESULTS

The evidence which has been accumulating within the past few years relative to the activity of the adrenal glands during shock would appear to indicate that these organs existed in a state of fatigue or at least that the output of epinephrin was not increased during or following the appearance of this syndrome. Thus Crile (13) reported in 1913 that there was no augmentation of output of epinephrin into the blood resulting from trauma under anesthesia. Also Bainbridge and Parkinson (5) and Corbett (15) conclude from a histological study of the adrenal glands that shock is the result of adrenal deficiency. These investigators based this assumption upon the decreased amount of chromaffin substance found in the glands at the time of examination.

Such findings are obviously in complete accord with that explanation of shock which holds that the condition is due to a splanchnic dilation of the blood vessels in the absence of epinephrin with the consequent fall in blood pressure.

The results of the experiments described in the present paper apparently are not in accord with those of the investigation just mentioned, nor are they in keeping with that conception of the cause of shock.

However, recently several investigators have reported work from which the conclusion might be drawn that a constriction of the arteries may occur in shock. Thus Muns (17) showed that during the development of shock, a peripheral constriction of the arteries of the limb occurred and if this was sufficient to offset the dilation in the splanchnic area, the pressure did not fall. Malcolm gives clinical evidence along similar lines.

It would appear that the apparent lack of harmony in the results arises from a failure to appreciate the actual conditions under which secretion occurs. Histological evidence does not necessarily give us an adequate picture of the functional activity of the cell. The former represents the condition of the physiological state at a single cross section of time; the latter must be represented over a considerable duration of time. Secretion is a double process. First, the material must be produced in the cell, and second, it must be eliminated into the blood or lumen of a duct. Normally these two processes occur simultaneously, but in conditions of disturbed cellular equilibrium, the one may overbalance the other; in which case the amount of substance in the cell at any one time holds no necessary relation to the

amount eliminated over a certain period of time. Thus the findings of Corbett (15), that the cells appear chromaffin poor, might very well have been interpreted as the result of more easy permeability of the cell for the hormone and while more than the normal amount was being produced, the material passed into the blood as soon as formation occurred. A similar explanation holds for the appearance at times of symptoms of hyposecretion with the glands containing an excess of the hormone. In this case the permeability for the substance was decreased, and the cell came into equilibrium with an excessive storage of the hormone, but with no elimination or perhaps a decreased one.

According to this point of view, the findings in this paper fit in with the histological picture. Increased production of epinephrin exists with a decreased amount present at any instant of time. It is difficult to bring Crile's (13) results into harmony with this conception. This investigator gives no details of his experiment, and it is well possible that the period of traumatization was not sufficiently prolonged in order to bring about the excessive discharge of epinephrin. In the present work there has never been a failure to find increased values of the hormone in the blood, if the handling of the intestines was prolonged for a sufficient period.

In 1905 Elliott (4) suggested that chromaffin tissue has the function of compensating for injury of sympathetic fibres and that after sympathetic impulses fail, their place is taken by the epinephric stimulation of the nerve endings.

The researches of Cannon and de la Paz (8) and Cannon and Hoskins (9) indicate that the adrenal secretion is largely a reserve for times of special stress. Hoskins and McClure (11) showed that there seems to be provided an adaptive distribution of the blood favorable to extreme muscular effort and that this is in some way related to epinephric action. Our results accord with this point of view. In shock the adrenals function as a line of secondary defense against a falling blood pressure. The presence of epinephrin in increasing amounts as shock progresses points to an attempt on the part of the circulatory system to redistribute the blood and bring about a peripheral constriction of the arteries wherever possible. In this way the normal pressure may be retained. If this self preservative mechanism is sufficient to offset the unknown factors which are instrumental in causing the fall in pressure, shock does not result fatally.

GENERAL CONCLUSIONS

1. That increased quantities of epinephrin are thrown into the blood during conditions of low blood pressure and shock.

2. That this increased amount of epinephrin found in the blood is accompanied by a hyper-activity of the adrenal gland and is not simply the result of the release of epinephric material stored in the gland.

3. That the epinephric content of the blood increases only after a somewhat prolonged continuation of the conditions leading to shock.

4. That the quantity of epinephric material in the blood increases with the prolongation of the period of low blood pressure and shock.

5. That this increased output of epinephrin into the blood may be a last effort on the part of the organism to resist the forces that are tending toward a fatal degree of low blood pressure.

BIBLIOGRAPHY

(1) Oliver and Schafer: Journ. Physiol., Proc. Physiol. Soc., 1894, xvi, 1.
(2) Oliver and Schafer: Journ. Physiol., Proc. Physiol. Soc., 1895, xvii, 9.
(3) Brodie and Dixon: Journ. Physiol., 1904, xxx, 476.
(4) Elliott: Journ. Physiol., 1905, xxxii, 401.
(5) Bainbridge and Parkinson: Lancet, 1907, i, 1296.
(6) Parkinson: Trans. Path. Soc., 1907, lviii, 187.
(7) Seelig and Lyon: Journ. Amer. Med. Assoc., 1909, lii, 45.
(8) Cannon and de la Paz: This Journal, 1911, xxviii, 64.
(9) Cannon and Hoskins: This Journal, 1911, xxix, 274.
(10) Falta and Priestly: Berl. klin. Wochenschr., 1911, xlviii, 2102.
(11) Hoskins and McClure: Arch. Int. Med., 1912, x, 343.
(12) Von Anrep: Journ. Physiol., 1912, xlv, 307.
(13) Crile: Journ. Amer. Med. Assoc., 1913, lxi, 2027.
(14) Janeway and Ewing: Ann. Surg., 1914, lix, 158.
(15) Corbett: Journ. Amer. Med. Assoc., 1915, lxv, 380.
(16) Janeway and Jackson: Proc. for Exper. Biol. and Med., 1915, xii, 193.
(17) Muns: Proc. for Exper. Biol. and Med., 1915, xii, 87.

BILE PIGMENT METABOLISM

VII. Bile Pigment Output Influenced by Hemoglobin Injections, Anemia and Blood Regeneration

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This paper shows the results obtained by the intravenous injection of hemoglobin in bile fistula dogs with and without anemia. Impressions gathered from various authoritative writings and published investigations led us to expect a pretty uniform reaction following an injection of hemoglobin in these bile fistula dogs under uniform conditions. We can not report any such uniformity of reaction and the tables given below require very careful study and conservative analysis. There are various stimulating possibilities which suggest themselves, but they must not be taken too seriously until the experimental data are conclusive. On the other hand, we must review some of the positive statements of other workers and suggest that a more conservative attitude be adopted toward the whole chapter of pigment metabolism.

The relation of hemoglobin injection to its elimination by the kidney has been studied by many investigators, recently by Pearce, Austin and Eisenbrey (1). They have studied the differences in hemoglobin elimination by the kidney when icterus was present or after removal of the spleen as compared with normal controls. There is a lowering of this threshhold of kidney elimination for hemoglobin when hemolytic or obstructive jaundice is present. Quite recently Sellards and Minot (2) have reported confirmatory work with human cases. They show that hemoglobin injection will cause hemoglobinuria in cases showing evidence of increased blood destruction, much more frequently than in normal controls. They state that this tolerance to hemoglobin bears no relation to the red cell count but to the amount of blood destruction in the body. The tolerance to hemoglobin

is low when blood destruction is high. We intend to suggest other factors which may be of importance in a consideration of this problem.

In these experiments with occasional exceptions we have used a unit amount of hemoglobin (obtained from 5 cc. of washed packed red cells) given into the jugular vein. This amount of hemoglobin as a rule will cause no hemoglobinuria and no clinical symptoms.

We must refer again to the long accepted theory that the pigment radicle of hemoglobin is quantitatively changed to bile pigment. This theory has been so long accepted that its very life tenure gives it a certain amount of respectability and perhaps more authority than it deserves. We would urge those who may be particularly interested to scrutinize the experimental evidence upon which this important claim is based. The best work is that of Stadelmann and his pupils (3). Their experiments were relatively few but carefully performed and accurately controlled. They used large amounts of hemoglobin, and followed the rise in bile pigment secretion over a long period. These workers do not claim that there is conclusive evidence for a quantitative elimination of the pigment radicle as bile pigment in bile fistula dogs.

No such conservatism marks the statements of Brugsch and Yoshimoto (4) and Brugsch and Kawashima (5) who claim that there is a quantitative elimination of bile pigments for a given amount of hematin injected. Further, that knowing the amount of bile pigment (or its end products) one can easily estimate the rapidity of hemoglobin disintegration! Careful scrutiny of their papers shows few experiments, fewer experimental data and no control of many important factors such as general condition, diet, icterus and hemoglobin determinations.

We insist that it should remain an open question whether the pigment radicle of hemoglobin is quantitatively eliminated in the bile as bile pigment. We believe there is more evidence against than for this view, but do not believe the question is settled.

Still more firmly do we insist that any estimation of blood destruction or of the life cycle of the red cell based upon the analysis of bile pigment or urobilin is absolutely faulty (Eppinger and Charnas (6), Wilbur and Addis (7) and others). We have shown that diet can increase bile pigment (8) and liver atrophy (Eck fistula) (9) or bile salts or bile fat (10) can decrease bile pigment secretion with no evidence of any blood changes. Now we suggest that the pigment portion of the destroyed hemoglobin under certain conditions may not be quantitatively eliminated as bile pigment. What information of

value concerning hemoglobin metabolism can we derive from an analysis of stercobilin alone? Diet may influence pigment elimination one way, liver function influence it another way and hemoglobin injection may cause a prompt rise in bile pigment output or a negative reaction. Can we state what has been happening in the liver from analysis of intestinal or bile pigments? Can we tell the life history of a cirrhotic liver from any analysis of the end product found at autopsy? To answer either of these questions is equally simple and requires the vision of a prophet.

When it was found that an Eck fistula liver put out much less bile pigment than a normal liver (9), we suspected that the lowered liver function of the Eck fistula was responsible. It was suspected that the liver might be concerned in *pigment construction*, that the liver might build bile pigment or even blood pigment. What substances can be built into various pigments? It is clear that blood feeding does not influence this bile pigment secretion (11). But might not pigment building be influenced in other ways? Can the liver use hemoglobin to form other pigments than bile pigments? If an anemia is produced does the liver take a part in the formation of this new blood pigment which goes on so rapidly? Given an anemia, what will the liver do with hemoglobin injected intravenously? There must be pre-pigment substances or parent pigments. Can such substances be held in reserve and used at times to form hemoglobin or other body pigments or thrown into the bile as bile pigments? Does the body build up this parent pigment substance only from "building stones" of the food or can it use pigment over and over again?

These and many other questions come up in any consideration of this complex problem of pigment metabolism. We have too long viewed this question of pigment metabolism with complacency, as perhaps one field in which our information was satisfactory. We urge that this whole question be approached with an open mind: that the simple little story of pigment katabolism from hemoglobin to urobilin be studied with care because this story may require revision.

EXPERIMENTAL OBSERVATIONS

These dogs with bile fistulae are comparable to those used in other experiments previously described. They had been under observation with a fixed routine for months and were in excellent condition. The methods used in operations, collections and analyses have been care-

fully described in the first paper of this series (12). There are no deviations from these published methods unless otherwise noted.

The preparation and injection of the hemoglobin solution was in all experiments done in a uniform manner. Blood freshly drawn from a dog was defibrinated or collected in oxalate, the red corpuscles washed repeatedly and packed firmly by the last washing in the centrifuge. Of these red cells 5 cc. were pipetted out, laked with distilled water and made up to 0.9 per cent solution with sodium chloride. This solution was given through a needle in the jugular vein. In no instance did this amount of hemoglobin given intravenously in bile fistula dogs cause any untoward results.

This amount of red corpuscles (5 cc.) should give approximately 60 mgm. of bile pigment, provided the pigment radicle of the hemoglobin is split off and excreted quantitatively as bile pigment.

Table 41 (dog 15–22) gives the results of several injections of hemoglobin into a simple bile fistula dog. This dog had been under observation for over a year in perfect condition under the usual uniform routine. If all the other experiments fell in with this one, we could make some generalizations which would indicate that the pigment metabolism is more simple than is probably the case.

The mean daily pigment secretion in this dog is pretty uniform, and we note the increase above this mean which follows the unit injection of 5 cc. laked red corpuscles. The urine contains a trace of bile pigment which increases after each injection of hemoglobin. The increase in bile pigment following the hemoglobin injection rises from 15 mgm. after the first to 38 mgm. following the fourth injection.

If hemoglobin injections showed this uniform graded increase from the first to the last injections or to a maximum output, we might assume a possible storing of pigment material in the body. When such storage capacity was exhausted, we might assume that the excess would be eliminated in the bile. This storage of pigment in one form or another is a possibility to be kept in mind but later experiments show the striking variations in the elimination of bile pigment after a unit injection of hemoglobin.

Tables 42 and 43 (also refer to table 74 in the following paper) are quite alike in their irregular reaction following a unit injection of hemoglobin. These dogs were in good condition and under the usual routine care. It is to be noted that dog 16–60 (table 43) has a subnormal mean bile pigment output. There are several possibilities to account for these low periods which will be discussed later. It will appear

TABLE 41

Normal bile fistula. Hemoglobin injection

Dog 15–22

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs*	
June 28	34	28	26		88	4.6	7.2	5.8		17.6		trace	33.5	
June 29	24*	30	15	14	59	5.4*	20.0	12.3	12.8	45.1	15.1	trace	33.8	
June 30	13	21	25		59	9.1	13.1	11.3		33.5		trace	33.8	
July 1					44					29.2		trace	33.8	Stools contain stercobilin
July 3	15	24	22		61	10.0	12.4	8.9		31.3		trace	33.5	Hemoglobin 120 per cent
July 5	19	15	19		53	12.0	9.1	8.5		29.6		trace	33.0	Hemoglobin 118 per cent
July 6	14*	22	14	24	60	6.7*	11.2	23.8	17.2	52.2	22.2	+	33.5	
July 7	32	24	17		73	8.0	8.1	6.5		22.6		trace	33.8	
July 8					22					25.6		trace	33.5	
July 10	9	20	16		45	10.8	18.0	9.7		38.5		trace	33.3	
July 11	17*	15	15	21	51	4.6*	17.0	23.6	21.8	62.4	32.4	+	33.5	
July 12	9	12	17		38	10.8	14.6	15.2		40.6		+	33.8	Red blood cells, 6,368,000. Hb. 117 per cent
July 13	16*	20	25	24	69	7.6*	22.0	19.0	27.0	68.0	38.0	+	33.8	
July 14	6	13	25		44	8.0	14.6	13.3		35.9		trace	33.8	
July 15					22					30.8		trace	33.8	
July 17					46					24.8		trace	33.8	

* 5 cc. laked red blood cells given intravenously at the end of the second hour.
Mean daily output in table = 30 mgm. per six hours.

that the reaction of this dog toward hemoglobin injections is much like the other animals. Variations all the way from 10 mgm. to 39 mgm. above the mean secretion of bile pigments are noted in these experiments. Moreover, too, injections only forty-eight hours apart may show striking differences, sometimes the first injection being fol-

TABLE 42

Control bile fistula. Hemoglobin injection

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
April 24	15	17	19		51	13.6	13.6	15.3		42.5		trace	36.3	Hemoglobin 106 per cent
April 25	22*	20	17	20	57	14.0*	18.8	19.2	19.8	57.8	18.3	trace	36.5	
April 26	18	18	19		55	13.8	11.3	15.9		41.0		trace	36.8	
April 27	17†	15	18	22	55	11.4*	18.2	18.8	18.4	55.4	15.9	+	37.0	
April 28	20	19	20		59	16.2	14.9	16.4		47.5		+	37.5	
April 29					48					29.8		trace	37.3	
May 1	14	14	16		44	11.6	15.2	18.8		45.6		trace	36.3	Hemoglobin 101 per cent
May 2	25†	24	18	17	59	16.4*	30.4	26.0	16.8	73.2	33.7	+	37.0	
May 3	21	22	15		58	11.5	11.9	10.4		33.8		trace	37.3	
May 4	16†	16	21	23	60	10.0*	22.2	21.8	18.9	62.9	23.4	+	37.0	
May 5	16	19	20		55	11.1	12.0	11.6		34.7		trace	37.3	
May 6					64					40.1		trace	37.0	
May 8	17	15	18		50	11.4	13.2	16.2		40.8		trace	37.0	Hemoglobin 116 per cent
May 9	20†	17	15	14	46	13.1*	20.6	22.4	15.8	58.8	19.3	+	37.0	
May 10	17	16	17		50	11.8	12.5	12.2		36.5		trace	36.5	
May 11	23†	21	24	24	69	11.9*	21.8	29.2	16.8	67.8	28.3	++	37.5	
May 12	24	19	18		61	15.2	12.8	16.2		44.2		+	37.5	
May 13					50					38.0		+	37.0	

* 4 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 39.5 mgm. per six hours.

Average of twelve control days before hemoglobin injection experiments was 35.9 mgm.

lowed by a greater increase in bile pigments than the second and vice versa. It is to be recalled that an injection of 5 cc. laked red corpuscles should yield 60 mgm. of bile pigment, if there is a quantitative elimination of the pigment radicle.

We do not wish to go into various possibilities at this time. We wish to emphasize this striking irregularity of bile pigment increase

TABLE 43

Control bile fistula. Hemoglobin injection

Dog 16-60

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS.	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 1	17	21	20		58	3.4	5.2	6.4		15.0		trace	37.5	Hemoglobin 94 per cent
May 2	21*	22	23	22	67	4.7*	11.4	18.9	15.8	46.1	23.3	++	38.0	
May 3	26	24	19		69	6.4	8.1	6.8		21.3		trace	38.5	
May 4	11*	20	14	26	60	6.7*	8.6	8.8	15.8	33.2	10.4	++	38.3	
May 5	25	24	23		72	7.3	4.9	6.2		18.4		+	38.5	
May 6					56					16.4		trace	38.0	
May 8	18	20	14		52	8.4	7.6	5.3		21.3		trace	37.5	Hemoglobin 93 per cent
May 9	26*	23	23	21	67	9.4*	17.0	20.1	19.2	56.3	33.5	++	37.5	
May 10	24	31	31		86	14.0	15.4	16.0		45.4		+	38.0	
May 11	25*	34	26	29	89	9.6*	20.6	23.6	18.2	62.4	39.6	+	38.5	
May 12	22	21	21		64	9.9	8.4	3.8		22.1		trace	37.5	

5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 22.8 mgm. per six hours.
Average of twenty control days before this experiment 22.4 mgm.

following a unit injection of hemoglobin in bile fistula dogs in apparent health. It is *possible* that the pigment radicle of hemoglobin is quantitatively eliminated as bile pigment when injected intravenously. If, however, the reaction following hemoglobin injection is as simple as usually assumed (merely a splitting off of the pigment radicle and elimination as bile pigment) why is not the reaction in these animals more uniform? In some experiments the crest of the wave of secretions seems to come in the third and fourth hours after injection with a return to normal during the total period of observation. Again there is much delay.

The reaction following bile or bile salt feeding is uniform under carefully controlled conditions (10). Why is not this reaction following hemoglobin injections more uniform? We believe that this reaction

is not simple and probably the whole body pigment metabolism is concerned. Some experiments given below strengthen this belief.

We realize that our six hour experimental period may be criticised, but there are more reasons for than against it. Such experiments must be repeated very many times because of individual variations in bile pigment secretion and obvious experimental variations. Twelve

TABLE 44

Bile fistula. Bleeding. Hemoglobin injection

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	16	17	17		50	11.1	13.9	15.2		40.2		trace	36.0	Hemoglobin 113 per cent. Bled 122 cc.
May 16	17	23	18		58	7.6	10.4	6.8		24.8		0	35.8	R. B. C. 6,444,000. Hemoglobin 114 per cent
May 17	16*	17	20	15	52	8.2*	13.7	19.8	17.0	50.5	14.7	+	35.8	
May 18	19	17	15		51	12.0	13.0	14.2		39.2		+	36.0	Stools contain no stercobilin
May 19	9*	25	20	15	60	5.8*	11.3	18.6	17.0	46.9	11.1	+	35.5	
May 20					44					39.2		+	35.0	

*5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 35.8 mgm. per six hours.

Average of twelve control days before hemoglobin injection experiments is 35.9 mgm.

or twenty-four hour collections are extremely trying for the dog and if repeated at short intervals will change the dog from a normal to an abnormal condition. As stated in our first paper, we are convinced that these short period collections give a more nearly normal picture than do the long or continuous observations where the dog can not even approximate a normal condition.

Tables 44 and 45 continue the observations on dogs 16–138 and 16–60 (tables 42 and 43). These tables show the effect of a slight bleeding before the injection of hemoglobin. The results of the hemoglobin injection are striking—there is only a small output of bile pigment above the mean in one dog and no bile pigment increase in the other dog. Here is a very suggestive observation which may have something

TABLE 45

Bile fistula. Bleeding. Hemoglobin injection

Dog 16–60

DATE	BILE										URINE. TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
May 15	22	22	21		65	8.9	9.9	8.9		27.7		trace	36.8	Hemoglobin 97 per cent. Bled 130 cc.
May 16	28	19	20		67	6.3	6.0	5.0		17.3		trace	37.3	R. B. C. 5,728,000. Hemoglobin 95 per cent
May 17	20*	15	13	12	40	3.8*	4.1	2.3	2.4	8.8	0	trace	37.8	
May 18	37	17	13		67	8.9	2.3	2.3		13.5		trace	37.5	Hemoglobin 96 per cent
May 19	27*	16	22	15	53	8.5*	6.4	5.4	3.4	15.2	0	trace	37.0	
May 20					60					19.6		trace	36.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 19.5 per six hours.
Average of twenty control days before this experiment is 22.4 mgm.

of importance back of it. May we assume that the greater part of this injected hemoglobin pigment radicle may have been deviated in the body to supply some pigment demand which was precipitated by this removal of blood? Before we answer *yes* to this question, as we might wish to do, let us consult the following tables 46 and 47 where the anemia is marked but the pigment retention not definite nor uniform—in other words, the anemic dogs react like the normal controls.

Tables 46 and 47 continue the history of these two simple bile fistula dogs. Anemia of a definite grade is produced by aspiration of blood from the jugular vein in sufficient amounts to cause a drop in the dogs' hemoglobin to 50 per cent or less. This acute demand for hemoglobin pigment to make into new red cells might react on the general pigment metabolism (compare tables 44 and 45) as has been suspected. There is no direct evidence in these tables 46 and 47. We note the same wide variation in bile pigment excretion following a unit injection of hemoglobin. In fact, the variations are even more marked than in the normal periods of these same dogs (tables 42 and 43).

The escape of bile pigments in the urine is more noticeable.

The mean daily bile pigment output during this anemia period (dog 16–138, table 46) is subnormal, 25.6 mgm. as compared to the normal 35.9 mgm. per six hours. This is much more evident during the period of regeneration following the acute anemia (table 48). Dog 16–60 has a subnormal output to begin with, and does not show this drop in bile pigment secretion (table 47).

Tables 48 and 49 show one important fact. During the period of acute regeneration following anemia produced by bleeding bile fistula dogs, there is a very low mean bile pigment output. Dog 16–138 has a normal mean daily output of 35.9 mgm. bile pigment per six hours (table 42). During the acute anemia period this mean output of bile pigment falls to 25.6 mgm. per six hours (table 46); but during the regeneration period following the anemia this mean bile pigment output per six hours falls to 11.2 mgm. or even to 7.2 mgm.—a drop to one-third normal or even less. Dog 16–60 shows a less striking reaction but one which is quite similar. It is to be recalled that this dog's initial mean bile pigment output per six hours was subnormal (table 43).

Dogs during the periods of *blood regeneration* following hemorrhage will put out less bile pigments than normal. There are several possible explanations and probably several factors concerned. The circulating red cells are somewhat below normal, and the number destroyed presumably smaller than normal, consequently the amount of pigment furnished in this manner is subnormal. It is more than possible that there is a conservation of pigment substances under these conditions of blood regeneration, but this term pigment conservation must be limited to the body pigments at present, however much the temptation to extend it to pigments introduced from without (e.g., hemoglobin). We have no evidence here that any more injected hemoglobin may

TABLE 46

Bile fistula. Anemia. Hemoglobin injection

Dog 16–138

DATE	BILE											URINE TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 22	31	25	30		86	12.5	11.9	16.4		40.8		+	34.5	Hemoglobin 115 per cent. Bled 122 cc.
May 23	17	15	20		52	9.6	11.8	14.9		36.3		trace	34.3	Hemoglobin 102 per cent. Bled 282 cc.
May 24	18	15	17		50	9.8	11.2	11.8		32.8		trace	34.5	Hemoglobin 94 per cent. Bled 200 cc.
May 25	16*	22	20	21	63	7.9*	19.6	24.2	18.6	62.4	36.8	++	34.3	Hemoglobin 66 per cent
May 26	20*	26	21	18	65	9.1*	23.6	19.8	15.4	58.8	33.2	++	36.0	
May 27	29	20	18		67	7.9	5.4	6.0		19.3		trace	36.3	
May 29	26	16	22		64	8.2	8.6	9.9		26.7		+	34.8	Hemoglobin 88 per cent. Bled 350 cc.
May 30	16	15	12		43	7.2	8.1	9.4		24.7		trace	34.8	Hemoglobin 63 per cent. Bled 200 cc.
May 31	19*	18	22	22	62	5.6*	14.5	33.6	15.8	63.9	38.3	++	34.5	Hemoglobin 52 per cent
June 1	20*	25	25	23	73	7.2*	13.5	15.8	14.0	43.3	17.7	++	34.8	
June 2	16	16	23		55	7.9	9.0	9.9		26.8		trace	34.3	
June 3					60					18.8		trace	34.5	Hemoglobin 71 per cent. Bled 350 cc.
June 5	18	18	19		55	8.9	9.4	8.5		26.8		trace	33.5	Hemoglobin 60 per cent. Bled 350 cc.
June 6	14*	19	18	14	51	4.7*	15.3	17.8	14.6	47.7	22.1	+	33.5	Hemoglobin 38 per cent
June 7	24	21	21		66	7.6	4.7	7.6		19.9		trace	34.3	
June 8	25*	25	24	32	81	5.1*	10.8	14.0	12.2	37.0	11.4	+	34.5	Hemoglobin 51 per cent
June 9	22	22	23		67	4.9	4.4	5.2		14.5		+	34.5	
June 10					52					20.0		+	34.0	Stools contain no stercobilin

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 25.6 mgm. per six hours.

Average for twelve control days with no anemia is 35.9 mgm.

TABLE 47

Bile fistula. Anemia. Hemoglobin injection

Dog 16–60

DATE	BILE											URINE TOTAL BILE PIGMENT, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 22	31	25	30		86	12.5	11.9	16.4		40.8		trace	36.3	Hemoglobin 94 per cent. Bled 130 cc.
May 23	23	18	24		65	9.9	9.4	11.9		31.2		trace	36.3	Hemoglobin 85 per cent. Bled 262 cc.
May 24	17	17	34		68	6.8	6.8	11.5		25.1		trace	35.8	Hemoglobin 70 per cent. Bled 100 cc.
May 25	16*	13	18	20	51	6.4*	7.3	13.8	17.3	38.4	17.3	trace	35.5	Hemoglobin 63 per cent
May 26	26*	28	34	27	89	8.8*	15.8	26.4	20.1	62.3	41.2	trace	36.3	
May 27	28	19	20		67	7.6	6.0	8.1		21.7		trace	37.8	
May 29	18	19	19		56	8.1	5.6	8.1		21.8		trace	35.8	Hemoglobin 78 per cent. Bled 325 cc.
May 30	24	12	19		55	5.9	4.4	6.4		16.7		trace	35.8	Hemoglobin 59 per cent. Bled 160 cc.
May 31	21*	23	24	21	68	5.2*	8.2	11.0	5.2	23.4	2.3	trace	35.3	Hemoglobin 51 per cent
June 1	22*	30	40	22	92	6.4*	8.2	20.8	8.9	37.9	16.8	+	36.0	
June 2	24	20	32		76	5.4	3.2	4.4		13.0		trace	35.3	Stools contain no stercobilin
June 3					50					17.0		trace	35.5	Hemoglobin 51 per cent
June 5	30	19	20		69	6.8	4.8	5.0		16.6		trace	34.5	
June 6	15*	20	28	27	75	6.0*	16.2	34.0	20.6	70.8	49.7	++	35.0	Hemoglobin 54 per cent
June 7	29	24	26		79	6.5	5.9	5.8		18.2		trace	35.3	Hemoglobin 62 per cent
June 8	30*	31	32	34	97	5.2*	14.0	22.3	16.0	52.3	31.2	++	36.3	
June 9	27	32	25		84	2.4	5.1	3.3		10.8		trace	36.0	

* 5 cc. laked red blood cells introduced into the jugular at the end of the second hour.
Mean daily output in table = 21.1 mgm. per six hours.
Average of twenty control days before anemia is 22.4 mgm

TABLE 48

Simple bile fistula. Anemia after-period. Hemoglobin injections

Dog 16–138

DATE	BILE: Amount in cubic centimeters: 1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	BILE: Bile pigments in milligrams: 1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean	URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS: Mixed diet
1916													*lbs.*	
June 26	29	27	27		83	2.0	2.4	1.8		6.2		0	33.5	Hemoglobin 83 per cent
June 27	20*	23	26	25	74	2.2*	9.9	10.5	10.8	31.2	24.0	trace	33.8	
June 28	24	25	23		72	3.2	2.8	2.6		8.6		trace	34.3	Feces contain no stercobilin
June 29	22*	23	25	22	70	3.0*	5.7	9.0	9.4	24.1	16.9	trace	34.1	
June 30	16	25	25		66	1.4	3.3	2.3		7.0		trace	35.0	

* 5 cc. laked red blood cells given intravenously at beginning of third hour.
Mean daily output in table = 7.2 mgm. per six hours.
Average for twenty control days during this anemia after-period, 11.2 mgm.

TABLE 49

Simple bile fistula. Anemia after-period. Hemoglobin injections

Dog 16–60

DATE	BILE: Amount in cubic centimeters: 1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	BILE: Bile pigments in milligrams: 1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean	URINE TOTAL BILE PIGMENTS, 6 HOURS	WEIGHT	REMARKS: Mixed diet
1916													*lbs.*	
June 26	37	24	32		93	3.3	1.7	2.8		7.8		0	34.0	Hemoglobin 76 per cent
June 27	19*	13	30	23	66	5.2*	3.8	10.0	10.4	34.2	24.3	0	34.0	
June 28	39	20	22		81	4.1	2.2	2.5		8.8		0	34.5	Feces contain no stercobilin
June 29	30*	21	28	30	79	5.2*	2.9	3.2	11.4	17.5	7.6	0	34.3	
June 30	39	31	33		103	5.9	3.5	3.7		13.1		0	35.0	

* 5 cc. laked red blood cells given intravenously at the end of the second hour.
Mean daily output in table = 9.9 mgm. per six hours.
Average of twenty control days in this anemia after-period is 13.0 mgm.

be retained in the body during active blood regeneration than during normal periods or intervals of acute anemia. It is probable that the body can not use hemoglobin as such when injected intravenously, but must break it down into its constituent parts before actual pigment construction can take place. How much of this indirect conservation of body pigment does actually take place we cannot say at present. There is at least a possibility that some of the hemoglobin

TABLE 50

Simple bile fistula. Hemoglobin injections
Dog 16–138

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
July 5	19	22	24		65	3.4	3.0	2.7		9.1		0	33.5	
July 6	16*	20	16	22	58	4.0*	5.4	6.1	7.9	19.4	4.4	0	34.0	
July 7	19	17	20		56	6.8*	7.7	8.1		22.6		0	34.0	
July 10	32	24	26		82	2.8	2.7	3.5		9.0		trace	33.8	Hemoglobin 91 per cent. R. B. C. 5,856,000
July 11	23*	15	18	20	53	3.6*	2.6	3.6	8.6	14.8	0	++	34.0	
July 12	23	22	22		67	9.4	5.4	5.4		20.2		trace	34.3	Stools contain no stercobilin
July 13	15*	15	31	19	65	2.0*	4.4	11.8	9.9	26.1	11.1	+++	34.5	
July 14	18	22	24		64	4.0	5.4	4.9		14.3		+	34.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table −15.0 mgm. per six hours.
Average for twenty control days during this anemia after-period is 11.2 mgm.

pigment radicle which does not appear in the bile in some of these injection experiments may be taken up by the body cells, digested and used for other purposes in the body. There is no evidence that the body cells acquire greater efficiency in this respect after repeated hemoglobin injections.

Tables 50 and 51 continue the observations and experiments upon these same two simple bile fistula dogs. Regeneration of red cells

following the anemia is still in progress and the general picture is very like that noted in tables 48 and 49. The very low bile pigment output of dog 16–138 after the usual hemoglobin injections is of interest. The urine contains more bile pigment than usual, but at the most only a very few milligrams. The level of blood hemoglobin has returned close to normal, but the mean bile pigment elimination is still much below normal—50 per cent of normal in one dog (16–138).

TABLE 51

Simple bile fistula. Hemoglobin injections

Dog 16–60

DATE	BILE											URINE TOTAL BILE PIGMENTS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
July 5	23	19	26		68	7.7	3.4	2.4		13.5		0	34.0	Hemoglobin 94 per cent
July 6	22*	26	23	23	72	6.4*	12.4	18.8	9.9	41.1	27.0	+	34.5	
July 7	24	19	21		64	6.7	4.3	4.7		15.7		+	34.3	
July 8					50					17.0		trace	34.0	
July 10	19	23	26		68	6.0	3.1	4.0		13.1		trace	33.3	Hemoglobin 102 per cent. R. B. C. 5,940,000
July 11	27*	26	23	25	74	4.2*	13.4	16.1	10.8	40.3	26.2	+	34.0	
July 12	25	12	32		69	6.1	3.5	7.2		16.8		+	34.3	Stools contain no stercobilin
July 13	19*	25	20	32	77	5.2*	6.7	9.9	10.8	27.4	13.3	+	33.8	
July 14	28	25	27		80	3.2	3.3	2.4		8.9		trace	33.8	

* 5 cc. laked red blood cells given intravenously at the end of the second hour.

Mean daily output in table = 14.1 mgm. per six hours.

Average of twenty control days in this anemia after-period is 13.0.

CLINICAL AND AUTOPSY SUMMARY

Simple bile fistula

Dog 16–60. Adult, fat, brindle bull dog, male, 44 pounds.

December 21, 1915. Ether anesthesia, bile fistula operation, general condition good during post-operative period.

May 1, 1916. Dog in good condition, 37.5 pounds. See table 43 for details—experimental period.

July 14, 1916. Dog in good condition—end of this experiment, 34 pounds.

July 21. General condition good, vigorous appetite, normal activity, 33 pounds. Hemoglobin 104 per cent.

Ether anesthesia and killed by bleeding.

Autopsy at once. Thorax negative. Spleen is normal size, the malphigian corpuscles are perhaps a bit enlarged. Liver shows a definite increase in brown pigment. Bile passages are clear and pale. Common duct is completely obstructed and no bile can enter duodenum. Its lower end is dilated to about 1 cm. in diameter. Stomach and intestines show a normal mucosa. The muscle coat of the small intestine is pigmented a maple sugar color which is characteristic of these dogs. Some of the retroperitoneal lymph glands are large and contain calcified foci. The other organs present nothing abnormal.

Microscopical sections are negative except from the liver, which shows a slight but definite increase in portal stroma which contains many mononuclear cells. There is a slight increase in pigment in the liver cells. There is no proliferation of the bile ducts.

Simple bile fistula

Dog 16–138. Adult bull dog, male, 38.8 pounds.

March 25, 1916. Ether anesthesia; bile fistula operation; general condition is excellent during post-operative period.

April 24, 1916. Dog in good condition, 36.3 pounds. See table 42 for details—experimental period.

July 14, 1916. Dog in good condition, 34.5 pounds.

End of experiments cited above.

August. Dog remains in good condition.

September 6. Dog in coma, bloody urine; blood shows very high urea and non-protein nitrogen.

Ether anesthesia and killed.

Autopsy at once. Thorax negative. Spleen is small and fibrous. Liver shows pale ducts and complete exclusion of bile from the duodenum. Stomach and intestines show nothing of interest. A urethral stone is found just below the prostate. Bladder is large and its wall is thick. There is a definite hemorrhagic cystitis with early ulceration. Both ureters and kidney pelves are dilated. The kidneys show multiple miliary abscesses in the cortex.

Microscopical sections add no information of importance to this experiment.

SUMMARY

It has been generally assumed from the publications of other workers that hemoglobin injection will be followed by a uniform elimination of a corresponding excess of bile pigment—in other words, hemoglobin injection is followed by its quantitative elimination as bile pigment.

Our experiments show that the reaction of a bile fistula dog under uniform conditions following a unit injection of hemoglobin intraven-

ously is certainly *not uniform.* That the elimination of the pigment radicle of the hemoglobin *may be quantitative* we can not deny, but surely all our experiments speak against any such probability.

Some hemoglobin injections may be followed by little or no increase in bile pigment secretion. It is possible that this pigment is taken up by the body cells and used for some purpose, perhaps to build other body pigments.

During periods of acute anemia from bleeding, the bile fistula dogs may show a fall in bile pigment output and at times a lessened elimination of bile pigment following a unit injection of hemoglobin. The latter is quite inconstant, and yet may indicate some conservation of hemoglobin either directly or indirectly. Compare the influence of splenectomy and the associated *increase* in bile pigments in the next paper.

Bile pigment elimination may be greatly depressed during the period of acute regeneration following an anemia, yet the response to hemoglobin injections may be even more irregualr and inconstant than under normal conditions. It is highly probable that we have a certain pigment conservation under these conditions, but the mechanism of this conservation may not be simple and direct but possibly very complex. The whole question of pigment construction is concerned in this problem and much more experimental work is required.

BIBLIOGRAPHY

(1) Pearce, Austin and Eisenbrey: Journ. Exper. Med., 1912, xvi, 375.
(2) Sellards and Minot: Journ. Med. Res., 1916, xxxiv, 469.
(3) Stadelmann: Der Icterus und seine verschiedenen Formen, Stuttgart, 1891.
(4) Brugsch and Yoshimoto: Zeitschr. f. exper. Path. u. Therap., 1910–1911, viii, 639.
(5) Brugsch and Kawashima: Zeitschr. f. exper. Path. u. Therap., 1910–1911, viii, 645.
(6) Eppinger and Charnas: Arch. f. klin. Med., 1913, lxxviii, 387.
(7) Wilbur and Addis: Arch. Int. Med., 1914, xiii, 235.
(8) Whipple and Hooper: This Journal, 1916, xl, 349.
(9) Whipple and Hooper: This Journal, 1917, xlii, 544.
(10) Hooper: This Journal, 1917, xlii, 280.
(11) Whipple and Hooper: This Journal, 1917, xlii, 256.
(12) Hooper and Whipple: This Journal, 1916, xl, 332.

BILE PIGMENT METABOLISM

VIII. Bile Pigment Output Influenced by Hemoglobin Injection; Splenectomy and Anemia

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This paper gives the results of experiments upon bile fistula dogs with an added splenectomy. Unit amounts of hemoglobin are injected intravenously during a control period, during an anemic period and during the period of blood regeneration. These experiments supplement those of the preceding paper as well as giving additional controls. The two bile fistula dogs are followed through a normal period, a period of bleeding and anemia, followed by an interval of acute blood regeneration. Here some peculiar differences due to the splenectomy are noted and will be discussed later.

A few points bearing on splenectomy and pigment production may be appropriately taken up in this place. It is known that splenectomy in dogs may often be followed by a moderate anemia (Musser (1), Pearce, Austin and Musser (2), Musser and Krumbhaar (3)), but it is not decided whether this anemia is due to blood destruction or to less active blood formation. It has been claimed by Martinotti and Barbaci (4) that after splenectomy the output of bile pigments drops to one-half normal. Enough experiments are reported below and in a preceding paper (5) to disprove this claim. We feel safe in asserting that under normal conditions a bile fistula dog will put out the same average amount of bile pigments whether with or without a spleen. It must be kept in mind that bile fistulae with splenectomy *plus* anemia from bleeding will often show remarkable periods of abnormally high pigment output alternating at times with periods of low bile pigment secretion.

This is a very interesting chapter in our splenectomy studies which calls for much more work. It is to be noted that the bile fistula dogs

with splenectomy may remain in a normal condition indefinitely and put out the usual amount of bile pigments per six hours. After a moderate anemia has been produced we may see a striking reaction—not a drop in mean bile pigment output as noted in controls with spleen intact but a period of great increase in pigment output. There is jaundice and heavy pigmentation of the urine. The blood cells may decrease still further and we may assume that all this increase in bile pigment secretion is due to red cell destruction. There may be another possibility or several more in fact.

We have stated that a bile fistula dog with splenectomy plus anemia may show periods of abnormally high bile pigment secretion during the long interval of blood regeneration which is greatly prolonged under such conditions. May we say that pigment production is depressed below normal? On the contrary we see an abnormal amount eliminated in the bile and urine (icterus). Further, it is to be noted that the color index of the blood during such periods is about unity as compared with simple bile fistula dogs with anemia when the color index is below one, as is usually observed in a secondary anemia. This may mean that pigment is present in excess, and the corpuscles are saturated with hemoglobin. It is not inconceivable that the frame work of the red cell may be formed through some mechanism and as it approaches maturity may be loaded with hemoglobin which it may select from the materials brought to it by the blood. Under some circumstances the formation of hemoglobin may be in excess of the stroma and the color index may approach unity. These experiments do not show many figures giving the blood color index, and we prefer to leave this point for future consideration.

How may we explain the periods of icterus which occur during the regeneration period following anemia in a bile fistula-splenectomy dog? We could show chart after chart in which periods of icterus appear suddenly with no obvious cause and as suddenly vanish with a long slow rise in the hemoglobin curve toward normal. There may be a sharp drop in the number of red corpuscles indicating disintegration of red cells to be in part responsible for the icterus. Yet the spleen is absent and cannot be blamed for this "hemolytic icterus." May we assume that the corpuscles manufactured during such periods are in some way faulty? We may believe that the stroma is less abundantly produced than the hemoglobin; perhaps the stroma is actually defective.

The hemoglobin injections give the same remarkable bile pigment fluctuations in relation to the mean output. It will be recalled that 5

cc. of laked red cells should be equivalent to 60 mgm. of bile pigment, if there is a quantitative change of the hemoglobin pigment radicle to bile pigment. Do we find any constant output of bile pigments above the mean following a unit injection of hemoglobin? On the contrary we find all variations from 4 mgm. to 42 mgm. increase above the mean daily bile pigment output—the same inexplicable variations

TABLE 61

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16-10

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3 4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3 4 hrs.	5-6 hrs.	7 8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
June 26	24	29	25		78	12.9	12.4	11.3		36.6		trace	29.0	Hemoglobin 106 per cent
June 27	27*	20	17	23	60	14.0*	14.9	8.8	26.8	50.5	17.5	+	29.0	
June 28	23	22	25		70	11.4	8.9	6.7		27.0		trace	29.1	Stools contain no stercobilin
June 29	18*	27	21	20	68	8.1*	17.0	17.1	16.1	50.2	17.2	+	29.0	
June 30	26	24	19		69	11.0	11.0	9.9		31.9		+	29.5	
July 1					46					31.8		trace	29.3	
July 5	25	21	19		65	12.9	9.4	8.6		30.9		trace	29.0	
July 6	21*	26	15	19	60	11.9*	15.4	19,6	18.8	53.8	20.8	+	29.5	
July 7	24	22	20		66	11.9	14.0	14.0		39.9		+	29.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 33 mgm. per six hours.
Average of twenty control days 34.4 mgm. per six hours.
Bile fistula operation and splenectomy done September 30, 1915.

in pigment output under as near uniform conditions as can be attained. The reaction following hemoglobin injection is the same in a simple bile fistula as with a combined bile and Eck fistula or a combination of bile fistula and splenectomy. The dogs used in these experiments had been under observation for months in apparent perfect health and under the usual routine described previously (6). The methods used have

been described in detail in former papers. These experiments are to be compared with those outlined in the preceding paper. These splenectomy animals were kept under the same routine as the bile fistula

TABLE 62

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16–27

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
April 24	11*	20	26		57	8.8	7.6	8.8		25.2		trace	25.8	Hemoglobin 103 per cent
April 25	18*	14	17	21	52	5.2*	6.9	12.7	9.4	29.0	4.3	trace	26.5	
April 26	15	25	25		65	6.8	7.9	7.3		22.0		trace	26.3	
April 27	26†	27	22	18	67	5.8†	12.8	14.0	5.6	32.4	7.7	trace	26.8	
April 28	23	18	25		66	7.9	8.9	10.2		27.0		0	26.5	
April 29					48					22.0		trace	26.0	
May 1	27	17	16		60	9.7	8.4	6.8		24.9		trace	25.5	Hemoglobin 87 per cent
May 2	14†	22	24	12	58	5.6†	20.8	18.9	8.2	47.9	23.2	++	26.5	
May 3	13	14	19		46	5.8	5.6	7.3		18.7		trace	26.8	
May 4	15†	20	17	23	60	11.5†	14.9	13.3	17.0	45.2	20.5	+++	26.8	
May 5	14	22	12		48	7.2	12.8	7.3		27.3		++	26.8	
May 6					38					23.8		+	26.3	
May 8	16	29	16		61	6.4	8.6	5.4		20.4		trace	26.3	Hemoglobin 86 per cent
May 9	12†	20	15	21	56	5.9†	17.0	7.4	4.9	29.3	4.6	++	26.0	
May 10	18	21	19		58	14.5	12.3	4.3		31.0		+	26.3	
May 11	19†	20	29	23	72	6.0†	7.2	19.4	16.4	43.0	18.3	++	26.3	
May 12	12	29	30		71	8.9	11.0	6.8		26.7		+	25.8	Stools contain no stercobilin
May 13					54					28.3		trace	26.0	

* 4 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 24.7 mgm. per six hours.

Average of twenty control days is 23.6 mgm. per six hours.

Bile fistula operation and splenectomy done December 8, 1915.

dogs without splenectomy, and the anemia was produced in the same way at the same time as can be seen by comparison of the various tables.

The three preceding tables (61, 62 and 63) show three bile fistula dogs with splenectomy, with normal blood hemoglobin and normal output

TABLE 63

Bile fistula. Splenectomy. Hemoglobin injection

Dog 16–41

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Tota 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
April 24	28	19	24		71	10.0	7.7	11.5		29.2		trace	36.0	Hemoglobin 111 per cent
April 25	19*	16	34	22	72	11.0*	12.9	26.0	13.4	52.3	27.3	+	36.3	
April 26	12	17	16		45	3.8	4.6	5.0		13.4		trace	36.8	
April 27	17*	23	40	32	95	3.1*	8.9	18.8	12.2	39.9	14.9	+	37.3	
April 28	36	40	33		109	9.6	11.6	11.2		32.4		trace	36.8	
May 1	18	16	19		53	14.4	12.0	10.4		36.8		trace	35.5	Hemoglobin 92 per cent
May 2	24*	22	25	32	79	9.8*	15.2	23.6	23.8	62.6	17.3	+	36.5	
May 3	36	29	20		85	22.8	18.2	16.1		57.1		trace	37.5	
May 4	32*	34	29	31	94	13.7*	26.6	30.4	30.8	87.8	42.5	+	37.5	Stools contain no stercobilin
May 5	23	19	29		71	12.4	12.8	17.0		42.2		+	37.0	
May 8	19	24	21		64	11.5	14.0	9.4		34.9		trace	35.8	Hemoglobin 103 per cent
May 9	21*	23	27	30	80	9.4*	18.8	18.9	8.8	46.5	11.7	+	35.5	
May 10	27	32	34		93	9.1	11.6	10.8		31.5		+	36.3	
May 11	35*	18	23	25	66	11.8*	9.4	18.8	19.0	47.2	12.4	+	36.5	
May 12	24	22	31		77	13.5	11.9	12.5		37.9		+	35.8	
May 13					46					35.2		trace	35.3	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 35 mgm. per six hours.

Average of twenty control days is 32.2 mgm. per six hours.

Bile fistula operation and splenectomy done December 2, 1915.

of bile pigments for the unit period of six hours. These experiments are to be compared with those in the preceding paper (tables 41, 42 and 43) in which splenectomy had not been performed. It will be noted that the total output of whole bile as well as of bile pigments is identical in the two groups of dogs. Splenectomy under these conditions does not influence the output of bile pigments.

TABLE 64

Bile fistula. Splenectomy. Bleeding. Hemoglobin injection

Dog 16-27

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs. Increase above mean			Mixed diet
1916												*lbs.*	
May 15	13	22	17		52	6.2	9.9	8.8		24.9	trace	25.5	Hemoglobin 90 per cent. Bled 85 cc.
May 16	17	17	24		58	5.7	6.5	9.8		22.0	trace	25.8	R.B.C. 4,880,000. Hemoglobin 83 per cent
May 17	12*	16	23	17	56	5.8*	9.0	12.4	8.8	30.2 6.2		26.0	
May 18	18	20	18		56	3.2	4.6	5.2		13.0	trace	26.0	Stools contain no stercobilin
May 19	15*	17	18	17	52	5.7	11.4*	16.2	17.6	45.2 21.2	++	25.8	
May 20					54					36.4	+	25.8	

* 5 cc. laked red blood cells injected into jugular at end of the second hour.
Mean daily output in table = 24.0 mgm. per six hours.
Average for twenty control days is 23.6 mgm.

Hemoglobin injections (5 cc. of laked red cells) are followed by the same fluctuations in bile pigment output whether a dog retains his spleen or has been deprived of its services. The same dog under identical conditions may respond to the hemoglobin injection with a rise of 11 mgm. bile pigment above the mean, again with a rise of 42 mgm.

Tables 64 and 65 show a continuation of the experiments with dogs 16–27 and 16–41 (tables 62 and 63). Each dog is bled a small amount from the jugular and later tested again with hemoglobin injections. These experiments are to be compared with the control non-splenec-

tomized dogs (tables 44 and 45). There are no particular differences noted in these tables (64 and 65) as a result of this initial bleeding. The dogs react to the hemoglobin injections as they did before the bleeding. Note particularly the figures in table 65 where it is seen that the first injection of 5 cc. hemoglobin caused a rise above the mean of only 3.4 mgm. bile pigment but two days later the same injection caused an increase of 38.4 mgm. bile pigment.

TABLE 65

Bile fistula. Splenectomy. Bleeding. Hemoglobin injection

Dog 16–41

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 15	14	16	24		54	9.8	8.2	15.8		33.8		trace	34.8	Hemoglobin 106 per cent. Bled 120 cc.
May 16	19	25	26		70	6.4	8.1	11.0		25.5		trace	34.5	R.B.C. 5,680,000. Hemoglobin 103 per cent
May 17	33*	30	41	28	99	6.7*	8.2	16.2	9.4	33.8	3.4	trace	34.8	
May 18	32	32	43		107	10.1	10.3	12.8		33.2		trace	35.0	Hemoglobin 100 per cent
May 19	14*	25	38	31	94	11.3*	22.6	26.6	19.6	68.8	38.4	trace	34.8	Stools contain no stercobilin
May 20					64					29.4		trace	34.8	

* 5 cc. laked red blood cells introduced into jugular vein at end of second hour.
Mean daily output in table = 30.4 mgm. per six hours.
Average of twenty control days is 32.3 mgm. per six hours.

The two preceding tables, 66 and 67, show some very unusual figures as compared with the control tables in the same dogs before the anemia, and compared also with the simple bile fistula dogs in the preceding communication. Table 66 shows a great rise in bile pigment output to double normal with persistent and increasing icterus. The hemoglobin falls after the bleeding has been discontinued, and remains low for weeks with alternating periods of icterus and high bile pig-

TABLE 66

Bile fistula, Splenectomy, anemia. Hemoglobin injection

Dog 16–27

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 22	15	22	20		57	8.1	15.2	10.3		33.6		trace	26.0	Hb. 90 per cent. Bled 85 cc.
May 23	7	16	15		38	8.4	21.4	14.8		44.6		+	25.8	Hb 81 per cent. Bled 215 cc.
May 24	19	17	17		53	12.0	12.7	14.5		39.2		+	25.8	Hemoglobin 59 per cent
May 25	17*	12	13	25	50	19.2*	15.2	17.2	37.4	69.8	16.7	++	26.0	Hemoglobin 58 per cent
May 26	17*	19	20	16	55	20.6*	27.4	32.4	28.8	88.6	35.5	++	26.8	Moderate icterus
May 27	22	17	17		56	21.8	18.4	20.6		60.8		++	27.3	Hemoglobin 36 per cent
May 29	17	14	19		50	16.8	16.7	19.8		53.3		++	26.0	Hemoglobin 28 per cent
May 30	15	17	11		43	14.4	19.2	14.4		48.0		+++	26.0	Definite icterus
May 31	11*	24	20	21	65	10.0*	29.2	30.6	23.8	83.6	30.5	+++	25.5	Hemoglobin 27 per cent
June 1	20*	21	19	17	57	11.0*	17.6	23.0	21.2	61.8	8.7	+++	25.3	Distinct icterus
June 2	23	15	15		53	20.4	17.0	17.1		54.5		++	24.8	
June 3					50					54.0		++	25.0	Hemoglobin 28 per cent
June 5	11	24	17		52	10.0	24.8	16.8		51.6		++	24.0	Hemoglobin 33 per cent
June 6	17*	19	21	17	57	15.2*	30.6	34.2	26.6	91.4	38.3	++++	24.3	Urine contains no urobilin
June 7	13	14	16		43	18.8	20.8	21.4		61.0		+++	24.5	
June 8	14*	16	16	17	49	16.4*	25.0	26.0	24.4	75.4	22.3	+++	24.3	
June 9	20	19	18		57	24.2	23.0	21.0		68.2		++++	24.5	R.B.C. 1,760,000. Hb. 31 per cent
June 10					24					68.8		++++	24.5	Marked icterus

* 5 cc. laked red blood cells injected into the external jugular at end of second hour.
Mean daily output in table = 53.1 mgm. per six hours.
Average of twenty control days before anemia 23.6 mgm.

TABLE 67

Bile fistula. Splenectomy. Anemia. Hemoglobin injection

Dog 16–41

DATE	BILE											URINE. TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
May 22	31	20	32		83	13.2	11.0	14.7		38.9		trace	34.3	Hemoglobin 101 per cent. Bled 120 cc.
May 23	20	16	15		51	14.4	10.0	6.8		31.2		trace	34.5	Hemoglobin 94 per cent. Bled 280 cc.
May 24	32	27	25		84	7.2	6.0	6.7		19.9		trace	35.0	Hemoglobin 71 per cent. Bled 100 cc.
May 25	18*	16	22	23	61	7.2*	10.7	19.6	10.4	40.7	11.0	trace	34.8	Hemoglobin 60 per cent
May 26	27*	28	31	27	86	7.9*	17.0	28.0	17.6	62.6	32.9	trace	35.3	
May 27	35	24	20		79	8.6	7.6	6.4		22.6		trace	35.3	Stools contain no stercobilin
May 29	25	13	15		53	15.2	10.5	16.2		41.9		trace	34.8	Hemoglobin 72 per cent. Bled 300 cc.
May 30	12	13	12		37	6.2	7.3	8.2		21.7		trace	34.5	Hemoglobin 60 per cent. Bled 160 cc.
May 31	27*	27	34	30	91	6.7*	8.5	21.4	12.8	42.7	13.0	+	34.3	Hemoglobin 48 per cent
June 1	49*	35	45	32	112	12.2*	11.0	22.6	17.2	50.8	21.1	++	35.3	
June 2	32	29	31		92	8.7	7.2	6.3		22.2		+	34.5	No icterus
June 3					88					33.6		+	34.8	Hemoglobin 57 per cent. Bled 250 cc.
June 5	23	22	22		67	10.9	9.4	10.9		31.2		+	33.5	Hemoglobin 46 per cent
June 6	14*	19	29	23	71	9.8*	22.0	37.6	22.8	82.4	52.7	+++	34.0	Hemoglobin 47 per cent. Urine contains no urobilin
June 7	22	22	21		65	12.8	14.0	14.4		41.2		++	34.5	R.B.C. 3,200,000. Hemoglobin 61 per cent
June 8	31*	30	27	28	85	14.0*	21.0	24.2	21.4	66.6	36.9	++	35.5	
June 9	35	31	29		95	12.5	9.1	7.2		28.8		+	35.8	Stools contain no stercobilin
June 10					78					24.2		trace	34.8	No icterus

* 5 cc. laked red blood cells introduced into the external jugular at the end of the second hour.

Mean daily output in table = 29.7 mgm. per six hours.

Average of twenty control days before anemia 32.2 mgm.

ment excretion followed by periods of blood regeneration and low bile pigment output.

The other dog (table 67) shows a less striking picture during this period, but in later periods showed even more striking icterus and high pigment output which interrupted the regeneration of red cells toward

TABLE 68

Bile fistula. Splenectomy. Anemia. Hemoglobin injection

Dog 16–27

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916												*lbs.*	
June 28	17	22	22		61	5.0	6.9	4.9		16.8		+	24.0	Red blood cells 3,542,000. Hemoglobin 61 per cent
June 29	10*	16	32	26	74	3.6*	5.4	15.8	10.5	31.7	25.0	+	24.5	Stools contain no stercobilin
June 30	19	27	23		69	4.8	4.2	2.6		11.6		trace	24.8	
July 1					62					7.0		trace	25.3	
July 3	15	28	22		65	4.4	5.6	4.0		14.0		trace	24.8	Hemoglobin 73 per cent
July 5	11	19	17		47	1.7	3.0	1.9		6.6		trace	24.5	
July 6	11*	16	19	20	55	2.0*	2.9	5.6	3.2	11.7	1.0	trace	24.8	
July 7	23	21	23		67	2.6	1.9	3.1		7.6		0	25.0	
July 8					46					12.4		0	25.3	
July 10	15	22	20		57	4.1	4.0	2.8		10.9		0	24.8	R.B.C. 3,880,000 Hemoglobin 78 per cent
July 11	11*	17	18	17	52	3.5*	8.0	25.0	11.4	44.4	33.7	++	24.5	
July 12	12	30	22		64	3.7	3.4	2.5		9.6		trace	24.8	Stools contain no stercobilin
July 13	15*	23	20	19	62	1.7*	6.2	11.6	10.8	28.6	17.9	+++	24.5	
July 14	12	25	24		61	3.8	4.5	2.7		11.0		++	24.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.

Mean daily output in table = 10.7 mgm. per six hours.

Average of twenty control days = 23.6 mgm. per six hours.

a normal count. This dog (16–41) required more prolonged bleeding to cause an anemia, but after some delay reacted very much like the first animal.

The reaction to uniform injections of hemoglobin (5 cc.) is truly remarkable, and ranges from 11 to 53 mgm. above the average mean

TABLE 69

Bile fistula. Splenectomy. Anemia

Dog 16–41

DATE	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			Mixed diet
1916										*lbs.*	
June 19	35	24	22	81	31.4	25.8	28.0	85.2	++	34.0	Hemoglobin 63 per cent. No icterus
June 20	29	25	26	80	35.2	39.4	40.2	114.8	++	33.8	
June 21	20	32	34	86	27.2	28.0	29.0	84.2	++	34.0	Urine contains no urobilin
June 22	27	25	30	82	30.6	27.0	32.4	90.0	++	34.5	
June 23	24	30	31	85	25.8	50.6	56.7	133.1	++	34.3	Feces contain stercobilin
June 24				82				125.6	++	34.0	
June 26	37	33	32	102	39.6	37.0	43.2	119.8	++	33.5	Hemoglobin 67 per cent
June 27	28	30	35	93	37.6	34.0	34.6	106.2	++	33.0	
June 28	30	33	32	95	25.1	28.6	23.8	77.5	++	33.5	Hemoglobin 69 per cent. R.B.C. 3,440,000
June 29	29	28	32	89	21.0	31.8	33.2	86.0	++	33.3	
June 30	26	28	28	82	37.6	40.2	35.2	113.0	++	33.5	
July 1				72				124.0	++	34.0	No icterus
July 3	36	33	31	100	40.8	46.0	50.0	136.8	+++	34.3	Hemoglobin 69 per cent
July 5	28	27	29	84	56.4	54.6	52.8	163.8	+++	33.8	
July 6	24	36	22	82	38.7	51.2	81.2	171.1	+++	33.8	Hemoglobin 42 per cent. R.B.C. 1,920,000
July 7	37	27	29	93	61.8	72.8	77.6	212.2	+++	34.0	
July 8				78				198.4	+++	33.8	Moderate icterus
July 10	24	25	22	71	64.8	78.8	69.2	212.8	+++	33.5	Hemoglobin 43 per cent. R.B.C. 1,720,000
July 11	25	23	24	72	58.4	77.4	78.8	214.6	+++	33.0	Urine contains no urobilin
July 12	22	32	28	82	59.6	86.4	75.6	221.6	+++	33.0	Distinct icterus
July 13	29	32	26	87	68.7	75.9	61.5	206.1	+++	33.5	
July 14	34	27	25	86	78.3	73.2	70.5	222.0	+++	33.5	Stools contain stercobilin
Average				85				146.3		33.7	

level. There is not even any parallelism with the amount of icterus, although the high figures in table 67 are noted in conjunction with icterus.

These two tables, 68 and 69, continue the observations upon same two dogs. The younger dog (16–27) has recovered from the spontaneous icterus (table 68), and shows a low bile pigment output, a rising hemoglobin curve and the same wide fluctuations in bile pigment excretion following a unit injection of hemoglobin. This same dog at later periods again developed spontaneous icterus with high bile pigment excretion and a drop in hemoglobin.

Table 69 (dog 16–41) shows a truly remarkable picture. Bile pigment is constantly present in considerable amounts in the urine and definite icteroid coloration of the skin and mucous membranes develops. The output of bile is normal, but the bile pigments are enormously increased—even more than *six times normal.* Note at this time the low hemoglobin curve and the color index which remains so constantly close to unity. It seems that this dog was putting out about the maximum amount of bile pigment, and perhaps he was manufacturing the maximum amount of pigment substance, which may account for the high color index of the blood.

CLINICAL AND AUTOPSY ABSTRACT

Bile fistula and splenectomy

Dog 16–10. Yellow, mongrel, female, 34.5 pounds.

September 30, 1915. Ether anesthesia; bile fistula and splenectomy. Condition remained unchanged after operation. Weight varied between 29 and 31 pounds.

June, 26, 1916. See Table 61 for details.

July 15, 1916. Dog in good condition except for mange. Ether anesthesia and killed.

Autopsy at once. Thorax negative. Liver is of normal size. There is an increase in pigment in the parenchyma. Bile passages are pale and clear. The duct is completely obliterated just above the duodenum. Mesenteric and portal lymph glands are slightly enlarged and a bit pigmented a yellowish color.

Bone marrow shows slight hyperplasia.

Kidney shows a little pigmentation of the cortex as seen in human cases of pernicious anemia.

Intestine shows a normal mucosa and considerable pigmentation of muscle coats which are of a maple sugar color.

Other organs are negative.

Microscopical sections. Liver shows definite increase in the portal stroma which in places contains nests of mononuclears. The bile ducts are normal. The liver cells close to the hepatic veins contain bile pigment in small and large colloid like grains and lumps.

Bile fistula and splenectomy

Dog 16–27. Young mongrel terrier, female, 23 pounds.

December 8, 1915. Ether anesthesia. Bile fistula operation and splenectomy. Condition excellent after operation. Weight varied between 23 and 26.5 pounds.

April 24, 1916. See table 62 for details.

July 14, 1916. End of experimental period; 24.8 pounds; condition constantly good until September, when there was some bleeding from the bile fistula.

September 30, 1916. Ether anesthesia and killed.

Autopsy at once. Thorax negative. Liver is deep greenish and much pigmented as may be seen with long standing obstruction; portal tissue not conspicuous, except in region of fistula, which is thickened and warty looking. Common duct is cut across and isolated from the duodenum. Intestines and pancreas show a definite pigmentation as described above. Lymph glands about pancreas are slightly enlarged and pigmented. Other organs are negative.

Microscopical sections. Liver shows a considerable increase in bile pigment deposited in the liver cells close to the hepatic veins. The portal stroma is very slightly increased and contains a few mononuclear cells. Bone marrow of femur shows a distinct hyperplasia which is explained by the period of bleeding during the two weeks preceding death. About one-half of the marrow is made up of fat cells. The other organs show nothing of importance.

Bile fistula and splenectomy

Dog. 16–41. Large normal bull dog, male, 38.5 pounds.

December 2, 1915. Ether anesthesia, bile fistula and splenectomy. Postoperative condition excellent.

April 24, 1916. See table 63 for details; 36 pounds.

July 14, 1916. End of this experiment, 33.5 pounds.

March 7, 1917. Dog in fair condition, 30 pounds.

Dog has shown repeated periods of spontaneous icterus and blood regeneration as tabulated above.

DISCUSSION

At present we can not offer a satisfactory explanation for these periods of icterus and high bile pigment elimination which are observed in bile fistula dogs with splenectomy. It is not certain how much of the bile pigment in dog 16–41 has been built up into hemoglobin and then broken down into bile pigment, but we can say that if this did happen, then the construction of red corpuscles must have been going on at a terrific pace. This dog over a period of two weeks put out on an average of 200 mgm. of bile pigment per six hours, over six times normal. We may safely calculate on a minimum output of 600 mgm. per twenty-four hours, which represents 50 cc. of red corpuscles. This dog weighed 33 pounds, and we may estimate his blood volume as 1200 cc. and dur-

ing normal periods about 600 cc. of red cells. His count at this time is two-fifths normal, and we must assume that he possessed about 240 cc. red cells. To account for this amount of bile pigment as coming only from the red cells, we must assume the destruction of his total red cells every four or five days. This is scarcely conceivable when we consider the slow regeneration of red cells which occurs after simple anemia in control dogs. This calculation does not take into consideration the pigment escaping into the urine.

Moreover, we wish to point again to the color index of unity which indicates probably a saturation of the corpuscles with hemoglobin. This fits in perfectly with the tentative suggestion that such animals are manufacturing pigment substance at a maximum capacity. Some of the pigment substance is formed into hemoglobin until the red cells can hold no more and some of the pigment substance escapes in the urine. Much of it escapes in the bile. We do not believe for an instant that all this pigment substance has been built up into hemoglobin and then degraded to other pigment substances. We do not believe the body is capable of doing this to such an extent as is shown in the last table, 69. There is evidently some extremely powerful stimulus present under these conditions of associated anemia, splenectomy and bile fistula which drives the body to maximum speed in its manufacture of pigments for the blood, bile and other tissues. We believe the liver is the mainspring in this mechanism. We hope to report in the near future on some of the interesting points which are suggested by these experiments.

SUMMARY

Splenectomy added to a simple bile fistula modifies in no way the secretion of bile pigments in the bile. A large number of experiments control this statement.

Hemoglobin injected intravenously gives no *constant* increase in the output of bile pigments in the bile. Splenectomy does not modify this reaction. Variations from 4 to 42 mgm. are noted following a unit injection.

Dogs with bile fistulae, anemia and an added splenectomy may show some remarkable deviations from the control experiments.

Experimental anemia in these bile fistula splenectomy dogs may give a very remarkable reaction—periods of spontaneous icterus, blood destruction and high pigment output may alternate with periods of regeneration and low bile pigment output without any demonstrable

cause. An interaction of the liver and spleen in the *construction* as well as in the *destruction* of the hemoglobin and red cell stroma may be indicated by these experiments.

Regeneration of red cells with consequent recovery from the experimental anemia is very greatly prolonged and may occupy months in the splenectomy experiments as compared with weeks in the simple bile fistula experiments without splenectomy.

The color index may remain uniformly high during this long period of blood regeneration in the splenectomy experiments. The output of bile pigments may average considerably above normal, and we may suspect an overproduction of blood and bile pigments with perhaps a deficiency of red corpuscle stroma.

BIBLIOGRAPHY

(1) Musser: Arch. Int. Med., 1912, ix, 592.
(2) Pearce, Austin and Musser: Journ. Exper. Med., 1912, xvi, 758.
(3) Musser and Krumbhaar: Journ. Exper. Med., 1913, xviii, 487.
(4) Martinotti and Barbaci: Morgagni, 1890, xxxii, 521, 593, quoted by Austin and Pepper, Journ. Exper. Med., 1915, xxii, 675.
(5) Whipple and Hooper: This Journal, 1917, xlii, 256.
(6) Hooper and Whipple: This Journal, 1916, xl, 332.

BILE PIGMENT METABOLISM

IX. Bile Pigment Output Influenced by Hemoglobin Injection in the Combined Eck-Bile Fistula Dog

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The combination of a bile fistula with an Eck fistula has been studied in an earlier communication (1). The Eck fistula shunts the portal blood directly into the vena cava, and limits the blood supply of the liver to the arterial blood coming through the hepatic artery. The Eck fistula liver is smaller than normal, shows central fatty degeneration and usually a subnormal liver function. The Eck fistula liver secretes less bile pigment than the normal liver—often 50 per cent of normal or less. There is strong evidence that bile pigment formation is dependent in part upon the functional activity of the liver and not solely upon the disintegration of red cells.

With these points in mind, we must consider carefully the tabulated experiments given below. Is there any evidence that the Eck fistula dogs have difficulty in supplying the usual amount of blood and body pigment as one might expect from the low bile pigment output? The Eck fistula dog shows periods of anemia which we suspect may be referable to an inadequate supply of pre-pigment material (manufactured in the liver?). This point can not be settled without much further work which we hope to supply in the near future. We have observed that removal of 150± cc. of blood from an Eck fistula dog may depress markedly the blood hemoglobin level while the same procedure in a control dog may influence very slightly the blood hemoglobin level. This applies to observations made twenty-four hours after bleeding. This observation may be explained in part by the experiments of Lamson (2) who demonstrated the storage of red cells held in reserve in the liver. This reserve, which is present in the normal liver, may not be found in the Eck fistula liver.

Combined Eck and bile fistula dogs have less tendency to develop icterus with bile pigments in the urine than do the simple bile fistula dogs. We have no evidence that the Eck fistula liver can not excrete the pigment radicle of hemoglobin as promptly as the normal liver. If the hemoglobin were not removed with some promptness from the blood stream, we might expect more formation of bile pigments in the tissue outside of the liver and more bile pigment in the urine. This is contrary to fact, as there is much less tendency to icterus and pigmentation of the urine in the Eck fistula dog than in the control.

The injection of hemoglobin intravenously in Eck fistula dogs gives much the same reaction as in simple bile fistula dogs. The unit injection of 5 cc. of laked red cells should give an increase of 60 mgm. of bile pigment above the mean, provided the pigment radicle of hemoglobin is quantitatively eliminated in the form of bile pigment. Compare the control table 74 with tables 71, 72 and 73 of the Eck fistula. It is seen that the variations in bile pigment output after hemoglobin injection are perhaps more pronounced in the Eck fistula than in the control. If anything, the Eck fistula dog can take care of more hemoglobin than the normal dog with less bile pigment showing in the urine and less bile pigment increase from the fistula. But one cannot be sure of this point when the same dog will put out an excess of 25 mgm. of bile pigment above the mean following a unit injection of hemoglobin and a few days later put out zero milligrams of bile pigment above the mean following the same unit injection of hemoglobin (tables 72 and 73).

EXPERIMENTAL OBSERVATIONS

The dogs used in these experiments were also used in experiments previously reported (1). Reference should be made to this paper for more complete details of control periods. The operative procedures have been reviewed. The general methods and experimental procedures have been described in detail in the first paper of this series (3).

The clinical history of each of these three dogs has been detailed in a recent publication (1), and need not be again outlined. The dogs were all in a uniformly good condition as regards weight, activity and diet. The tables give all experimental data.

The three preceding tables show several points of interest (tables 71, 72 and 73). The same dog with combined Eck and bile fistula shows a slow gain in hemoglobin from 38 per cent to 98 per cent during a period of three and one-half months. During the anemic period

(table 71) we see a marked reaction to a unit injection of hemoglobin. One injection of 5 cc. laked red blood cells given intravenously causes a rise in bile pigment secretion of 24 mgm. The curve of secretion is very sharp, and the apex falls usually during the second two-hour period after hemoglobin injection, the final period often showing a return to normal. The urine after such hemoglobin injections at the most shows a trace of bile pigment. The Eck fistula animals on the

TABLE 71

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs. Increase above mean			Bread, bone and milk diet
1916												*lbs.*	
March 27	15	12	18		45	2.3	4.4	4.0		10.7	trace	20.3	Hemoglobin 45 per cent
March 28	21*	14	13	9	36	3.3*	10.4	13.4	10.2	34.0 24.3	trace	20.5	
March 29	21	18	15		54	3.3	2.4	3.0		8.7	trace	20.0	
April 3	25	21	20		66	3.9	2.9	2.2		9.0	trace	20.8	Hemoglobin 38 per cent
April 4	14†	18	16	6	40	2.2†	2.4	3.2	2.0	7.6 0	trace	21.3	
April 6	22	22	18		62	4.9	4.4	4.0		13.3	trace	20.3	
April 7	25	16	18		59	2.3	3.1	1.9		7.3	0	20.8	

* 5 cc. laked red blood cells given intravenously at end of second hour.

† 5 cc. laked red blood cells given intramuscularly at end of second hour.

Mean daily output in table, 9.8 mgm. per six hours.

Average output for 30 control days is 11.8 per six hours.

Eck fistula operation September 13, 1915. Bile fistula operation February 17, 1916. Excellent condition throughout observations.

whole seem to store away the injected pigment more effectually than the simple bile fistulas. This statement is made with reservation in the face of wide fluctuations following hemoglobin injections in simple bile fistula dogs and bile fistula-Eck fistula dogs.

The after anemia period (table 73) shows even less reaction to hemoglobin injections. Two injections are followed by no bile pigment increase and one injection causes a rise of 19 mgm. in bile pigment

secretion. We get no evidence in any of these experiments that the Eck fistula liver can not eliminate the pigment radicle as promptly as the normal liver following hemoblogin injections. If anything, the Eck fistula liver seems to react more promptly than the normal liver in pouring out any excess of bile pigments which may be formed as a result of hemoglobin injections.

TABLE 72

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs. Increase above mean			Bread, bone and milk diet
1916												*lbs.*	
June 5	26	18	12		56	2.4	2.0	0.8		5.2	0	21.0	Hemoglobin 74 per cent
June 6	19*	17	17	16	50	2.1*	6.1	16.8	12.0	34.9 27.0	trace	20.9	
June 7	20	11	8		39	8.6	4.4	1.6		14.6	trace	21.0	
June 8	16†	13	7	4	24	4.0†	7.0	10.2	2.1	19.3 11.4	trace	21.5	Hemoglobin 78 per cent. R. B. C. 5,352,000
June 9	15	11	18		44	1.7	0.7	2.0		4.4	0	21.8	
June 10					48					5.4	0	21.3	
June 12	15	15	12		42	2.6	3.0	1.1		6.7	0	20.8	Hemoglobin 72 per cent
June 13	16†	14	14	11	39	4.0†	7.9	8.2	4.4	20.5 12.6	trace	20.5	
June 14	13	6	5		24	8.6	2.0	0.6		11.2	0	20.3	
June 15	9†	12	16	12	40	6.8†	9.8	16.4	7.0	33.2 25.3	trace	20.3	
June 16	15	7	5		27	5.0	1.9	1.0		7.9	trace	20.5	

* 7.5 cc. laked red blood cells given intravenously at end of second hour.
† 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table — 7.9 mgm. per six hours.
Average output for thirty control days is 11.8 mgm.

Another fact deserves a word. It is to be noted in tables 72 and 73 that the color index of this dog is constantly low. Other periods show a very striking deviation from normal, and there is some evidence that the red cells contain a subnormal amount of hemoglobin. A similar condition may be found in simple bile fistulae with anemia, but it is

not as marked nor does it persist with a high red count as we have observed in the combined Eck-bile fistula. We may suspect that this lack of hemoglobin in the red cells may be due to inefficient pigment production in this dog. This point calls for much more work, and we believe more study should be directed toward pigment *construction*

TABLE 73

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16–15

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	1–2 hrs.	3–4 hrs.	5–6 hrs.	7–8 hrs.	Total 6 hrs.	Increase above mean			Bread, bone and milk diet
1916													*lbs.*	
June 19	17	15	22		54	4.2	3.4	7.4		15.0		0	20.0	Hemoglobin 78 per cent
June 20	12*	12	11	8	31	5.4*	7.9	14.4	9.2	31.5	19.2	trace	20.5	
June 21	7	7	6		20	12.8	8.4	4.5		25.7		trace	19.9	
June 26	17	12	13		42	2.3	1.9	0.9		5.1		0	20.5	Hemoglobin 80 per cent
June 27	10*	11	9	8	28	3.6*	5.0	1.6	1.2	7.8	0	0	20.3	
June 28	13	9	8		30	2.9	1.2	0.8		4.9		0	20.5	Feces contain stercobilin
July 3	16	13	9		38	3.6	2.6	1.2		7.4		0	20.5	
July 5	20	15	14		49	2.8	1.7	1.3		5.8		0	20.3	Hemoglobin 98 per cent. R. B. C. 6,332,000
July 6	14*	11	9	10	30	4.1*	5.7	5.4	4.9	16.0	3.7	0	20.5	
July 7	9	7	11		27	7.3	6.6	2.7		16.6		0	20.3	
July 8					28					18.2		0	20.5	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table — 12.3 mgm. per six hours.
Average output for thirty control days of this period, 11.8 mgm.

rather than pigment *destruction*. We hope to submit experiments which may give information concerning pigment construction in the body.

Dog 16–146 (table 74) acts as control to the three preceding Eck fistula tables. This control dog is of about the same weight, activity

and temperament as the Eck fistula dog (16–15). Both the control dog and the second Eck fistula (table 75) show much variation in bile pigment secretion following the unit injection of hemoglobin.

TABLE 74

Simple bile fistula control. Hemoglobin injection

Dog 16–146

DATE	BILE											URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams								
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	Increase above mean			Mixed diet
1916													*lbs.*	
June 5	11	16	18		45	6.4	7.6	6.8		20.8		0	17.5	Hemoglobin 84 per cent
June 6	16*	14	21	18	53	5.4*	17.0	21.8	14.5	53.3	33.5	+++	17.5	Urine contains no urobilin
June 7	14	17	17		48	3.8	6.1	6.1		16.0		trace	17.8	
June 8	14*	16	19	13	48	5.4*	13.2	17.1	7.3	37.6	17.8	++	18.3	
June 9	17	16	14		47	6.8	7.2	5.4		19.4		trace	18.3	
June 10					36					14.8		0	18.2	
June 12	16	17	16		49	8.6	7.6	7.6		23.8		0	17.8	Hemoglobin 90 per cent
June 13	9*	13	19	15	47	5.1*	13.4	17.8	10.4	41.6	21.8	++	18.0	
June 14	11	14	14		39	7.6	6.9	6.9		21.4		trace	18.3	
June 15	16*	19	17	17	53	8.6*	15.6	16.0	14.4	46.0	26.2	++	18.5	
June 16	12	12	11		35	8.2	9.2	7.0		24.4		trace	18.3	Stools contain no stercobilin
June 17					42					18.8		trace	18.0	
June 19	20	17	15		52	6.8	6.5	7.2		20.5		trace	18.0	Hemoglobin 89 per cent
June 20	13*	16	14	15	45	3.8*	9.7	12.8	12.0	34.5	14.7	trace	17.8	
June 21	14	12	16		42	6.4	6.4	5.4		18.2		trace	17.5	

* 5 cc. laked red blood cells given intravenously at the end of second hour.
Mean daily output in table = 19.8 mgm. per six hours.
Average of twenty control days is 18.1 mgm.

Table 75 (dog 16–139) shows an unusually high mean bile pigment output, in fact, practically normal for a control dog of equivalent weight. It can be seen in an earlier communication (1), table 36, that this same dog in the previous month put out 14.2 mgm. per six

hours. After the hemoglobin injections were begun, there is a constant high level of bile pigment elimination. We do not believe that one depends upon the other, but must admit that there may be some possible connection. Secondly, it is to be noted that this dog devel-

TABLE 75

Eck fistula. Bile fistula. Hemoglobin injection

Dog 16-139

DATE	BILE										URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters					Bile pigments in milligrams							
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	Total 6 hrs. Increase above mean			Bread, bone and milk diet
1916												*lbs.*	
May 31	8	12	11		31	5.8	6.4	5.0		17.2	trace	29.0	Hemoglobin 86 per cent
June 1	9*	9	11	11	31	4.9*	3.6	11.4	10.0	25.0 0.6	trace	29.0	
June 2	10	12	14		36	9.2	8.2	5.4		22.8	trace	29.3	
June 5	13	13	11		37	10.5	9.4	5.9		25.8	trace	28.8	Hemoglobin 88 per cent
June 6	7*	10	10	10	30	6.6*	10.4	16.2	15.0	41.6 17.2	++	29.3	Urine contains no urobilin
June 7	6	9	12		27	7.2	8.0	6.7		21.9	+	29.3	
June 8	14*	12	11	9	32	11.2*	9.7	12.2	11.6	33.5 9.1	++	29.5	Hemoglobin 82 per cent. R. B. C. 5,416,000
June 9	7	12	13		32	9.2	11.9	8.8		29.9	trace	29.5	
June 10					30					25.8	trace	29.8	
June 19	14	13	12		39	6.4	6.2	6.2		18.8	trace	29.0	Hemoglobin 91 per cent
June 20	17*	17	14	15	46	6.8*	13.0	17.0	16.2	46.2 21.8	++	29.0	
June 21	13	12	14		39	12.8	10.8	9.4		33.0	++	28.8	
June 22	7*	11	11	12	34	16.0*	20.0	15.0	21.2	56.2 31.8	++	29.0	

* 5 cc. laked red blood cells given intravenously at end of second hour.
Mean daily output in table = 24.4 mgm. per six hours.
Mean daily output in month previous, 14.2 mgm. per six hours.

oped frank clinical distemper within two weeks after this experiment ended, and it is possible that the disease was latent in this animal during this period of experimentation. Distemper is an infection in dogs which can cause a variety of unusual complications.

SUMMARY

We have submitted evidence that the Eck fistula liver secretes less bile pigment than the control liver. It is possible that bile pigment formation is dependent in part upon liver function rather than upon the disintegration of red cells.

It is probable that the Eck fistula liver can eliminate hemoglobin from the blood stream as promptly as the control liver.

The Eck-bile fistula dog has less tendency to icterus and staining of the body tissues with bile pigment. Even with a high red cell count the color index will often be low in the Eck fistula. This is evidence to show that the Eck fistula dog has a subnormal pigment building capacity.

The Eck-bile fistula dog shows the same great fluctuation in bile pigment excretion following a unit injection of hemoglobin. It is possible that these dogs can store more pigment substance than the control dogs after unit injections of hemoglobin.

BIBLIOGRAPHY

(1) Whipple and Hooper: This Journal, 1917, xlii, 544.
(2) Lamson: Journ. Pharm. and Exper. Ther., 1915, vii, 169.
(3) Hooper and Whipple: This Journal, 1916, xl, 332.

THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

II. Volume Changes and Venous Discharge in the Spleen

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In the first paper of this series (1) attention was called to the desirability of further investigation of the effects of adrenin on the blood-flow in various organs with adequate attention to dosage and duration of administration of the drug.

Apparently in previous studies along this line the spleen has received relatively little attention. Oliver and Schaefer (2) were the first to investigate the effects of suprarenal extract in this organ. In the several cases studied the reaction to the extract was an "enormous" contraction. In none was any dilatation observed except for a short time preceding the reaction proper. This was regarded as probably a passive effect. Bardier and Frenkel (3) recorded the results of a study on a single animal, which was, apparently, under the influence of curare. Their extract was made by macerating desiccated or fresh gland for twenty-four hours at 37°C. After three injections they noticed: (a) Dilatation for three minutes followed by constriction while systemic blood pressure rose from 110 to 220 mm.; (b) Contraction followed by dilatation while the systemic pressure varied between 100 and 120 mm.; (c) Dilatation followed by contraction while the arterial pressure varied from 80 to 180 mm. Judging from the initial arterial pressures in each case the animal was relapsing into shock. The vasomotor reactions in the first and third cases indicate that the dosage transcended physiologic limits. What part was played in the reactions in the spleen by the curare and by protein decomposition products in the extracts was not determined. Falta and Priestley (4) observed that the spleens of animals exposed several hours after subcutaneous injections of large doses of adrenin appeared anemic. Vincent (5) without giving any details states that adrenin administered intravenously causes a

contraction of the spleen. All the foregoing observations indicate that the outstanding effect of adrenin in the spleen is a marked contraction but both primary and secondary dilatations have been recorded. We have extended the investigations as herein reported.

Technique. In all cases dogs have served as experimental animals. In most instances ether or morphin-ether anesthesia was employed but a few were decerebrated. The experimental results in both cases were similar. In some instances the vagi were cut but this procedure made no apparent difference in the outcome. The adrenin (Parke, Davis "Adrenalin") was introduced into a femoral vein, sometimes instantaneously and sometimes slowly. Various dosages were used depending upon whether pressor or depressor effects were desired. Simultaneous records were made of changes of splenic volume or venous outflow or both and of changes of femoral arterial pressure.

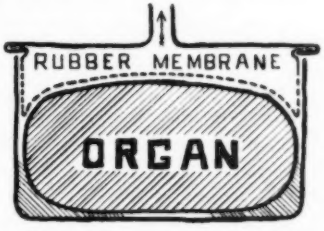

Fig. 1. Diagram showing construction of plate plethysmograph.

Various types of plethysmographs were tried, including a Roy's oncometer. The most satisfactory type was one improvised in the laboratory; it is somewhat like that of Edmunds. The general plan of the apparatus is shown in figure 1. A box was selected of suitable size to contain the organ under investigation. For this purpose a celluloid soap box proved satisfactory. Such boxes are light, inexpensive and easily procurable at any shop where toilet accessories are sold. The volume changes were transmitted by means of a plate plethysmograph which served as a lid for the box. It was made as follows: A sheet of copper, gauge 24, was cut the same shape as the top of the box and about 2 cm. greater in diameter. A sheet of rubber tissue was then cut in turn 2 cm. greater in diameter than the copper plate. A hole was drilled through the middle of the plate to communicate with a brass tube 3 mm. in diameter soldered to it to form the outlet of the plethysmograph. The edges of the plate were then bent up around the four sides to make a shallow box about 0.5 cm. deep. The margin was incised at intervals to permit this bending. Melted collophonium wax or marine glue was introduced as a narrow zone around the outer edge of the box. This served to affix the rubber membrane which was next introduced. The excess circumference of the membrane was taken up in a series of pleats

which were held in place by bending inward and down the sides of the box. A marginal zone of glue or collophonium wax applied just before the copper was bent down served to make the joint hermetically tight. In case this result was not secured at the first attempt a bit more of the cement was introduced to stop the leak or else the rim at that point was simply held for a moment in hot water to melt the cement already applied and allow it to flow into the crevice.

In using this type of plethysmograph the organ is isolated and placed on a wet cotton pad in the box. A slit is cut in the side of the box to accommodate the blood vessels of the organ. The plate plethysmograph is then applied as a lid to the top of the box and secured in place by two rubber bands. It is then attached by rubber tubing to a recorder. A T-tube interpolated in the connecting tube permitted the inflation of the plethysmograph with air under a slight pressure sufficient to cause the membrane to adapt itself to the inclosed organ but not great enough to interfere in any way with the circulation.

In a few instances the Cushny cardiograph was found to give satisfactory results in recording changes in the dimensions of the organ but its use involved a greater degree of exposure of the viscera than did the plethysmograph.

Venous outflow was recorded by drops from an oiled cannula tied in a relatively small vessel. In some cases the spleen was divided and volume change and outflow determined simultaneously.

The float recorder previously described (1) was employed in all cases.

Results. In determining the effects of adrenin in the spleen 17 dogs were used. In the plethysmograph studies 65 injection and 18 infusion experiments were made while the venous outflow series included 34 injection and 20 infusion experiments.

In nearly all cases the effects on organ volume were similar to those described by Oliver and Schaefer for pressor injections, namely, a brief, supposedly passive dilatation followed by marked contraction. The same results were also obtained with depressor injections and infusions as well as with pressor infusions. Figure 2 which was obtained with a slightly depressor infusion will suffice to illustrate all these cases. It was found to be possible to hold a spleen in a state of uniform contraction by adrenin infusion for ten minutes. Longer periods were not tried. The effect in the spleen lasted from a half to five minutes after blood pressure had returned to normal. In no case with either large or small dosage was a secondary dilatation observed during an infusion period.

Occasionally, however, as shown in figure 3, a dilatation occurred after the administration of the adrenin was discontinued. This effect was not passive since it was noted when the arterial pressure was either normal or depressed. With no dosage was a pure splenic dilatation observed.

The threshold for the reaction in the spleen was highly variable but generally it was lower than that for changes of arterial pressure. In the most sensitive preparation investigated splenic contraction first appeared with an injection of 0.5 cc. of a 1:2,000,000 solution. This means of course that the spleen is one of the most sensitive organs in the body and reacts before a sufficiently widespread effect occurs to influence the general blood pressure.

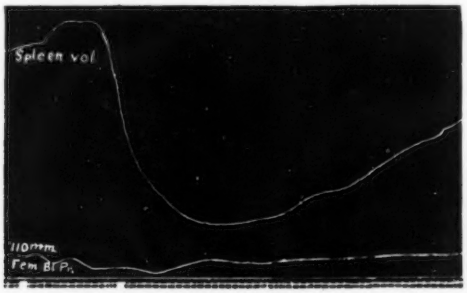

Fig. 2. Spleen contraction under the influence of adrenin, depressor infusion. Dose 2.8 cc. 1: 200,000 in 65 seconds. Dog weight 15 kilos. Time, five seconds.

In several instances spleens that were previously quiescent began to undergo rhythmic changes in volume after the injection of adrenin. In such cases a new injection would check the rhythmic contractions but they would begin again as the effect of adrenin wore off.

The effect of adrenin on venous outflow was just what would be expected from a consideration of the volume changes. A typical graph is reproduced as figure 4. During the preliminary dilatation period the flow was augmented. The augmentation persisted during the first part of the contraction period as the blood already in the organ was being expelled. Then during the remainder of the contraction period the flow was depressed, reaching the normal rate at about the same time splenic volume was restored to normal. The depressed outflow during the latter part of the period was obviously due partially to retention of the blood in the expanding organ.

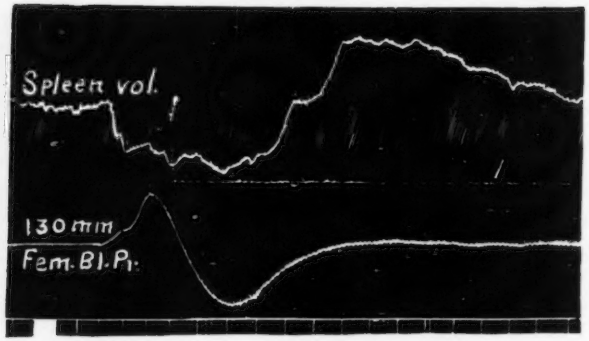

Fig. 3. Shows secondary dilatation of the spleen after injection of adrenin, 1 cc. 1: 50,000. Time, five seconds.

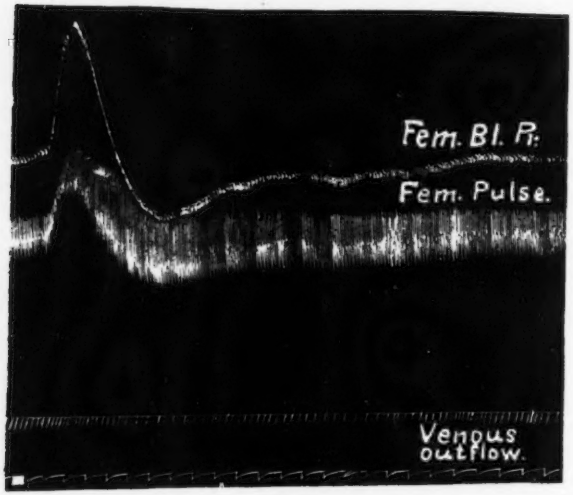

Fig. 4. Shows effect of injection of adrenin, 1: 100,000, on outflow from splenic vein.

SUMMARY

Adrenin in all effective dosages whether injected instantaneously or infused causes in the spleen a brief dilatation followed by a contraction.

Occasionally the contraction is followed by a secondary dilatation after the administration of the adrenin is discontinued.

Aside from the brief preliminary effect in no case was a pure (primary) dilatation observed.

The threshold for splenic contraction is lower than for changes in arterial pressure.

Occasionally a quiescent spleen is stimulated by adrenin to rhythmic contractions.

Adrenin causes a brief increase, then a decrease in the outflow from the splenic veins.

BIBLIOGRAPHY

(1) Hoskins, Gunning and Berry: This Journal, 1916, xli, 513.
(2) Oliver and Schaefer: Journ. Physiol., 1895, xviii, 231.
(3) Bardier et Frenkel: Journ. d. Physiol. et d. Pathol. Gén., 1899, i, 950.
(4) Falta und Priestley: Berl. klin. Wochenschr., 1911, xlviii, 2102.
(5) Vincent: Internal secretions and the ductless glands, London, 1912, 164.

THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

III. Volume Changes and Venous Discharge in the Kidney

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The effects of adrenin on vascular conditions in the kidney, as in several other organs, were first studied by Oliver and Schaefer (1). Without going into details as to any possible differential effects of large and small doses, of the effects of long continued administration of the extracts or of after effects, they reported that suprarenal extracts cause a marked diminution in kidney volume. Largely on the basis of this fact and the observation that the spleen is similarly affected the generalization was offered that such extracts cause a marked vasoconstriction throughout the splanchnic area.

Four years later, in 1899, Bardier and Frenkel (2) investigated the relation of suprarenal extracts to diuresis but included also in their study the vasomotor effects. Their experiments were made on anesthetized dogs, apparently under the influence of curare. Their extracts were made either from desiccated glands or from fresh glands macerated for twenty-four hours at body temperature. Judging from the effects on arterial pressure relatively large doses were employed. The extracts were administered intravenously. These authors described as typical effects a contraction of the kidneys and a depression of urine flow followed by a dilatation of the organ and a diuresis. In certain exceptional cases, however, the injections were followed at once by dilatation and polyuria. Whether the use of curare or the presence of protein decomposition products in their extracts played any part in the results was not determined.

In the same year Gottlieb (3) included the kidneys in a series of experiments on the effects of adrenal extracts on the heart and blood vessels. He worked on isolated kidneys of hogs and dogs. It was noted that when such extracts were added to the perfusate that was being

passed through the organs a marked decrease in outflow resulted. This finding was corroborated by Gioffredi (4). Similar experiments were made by Sollmann in 1905 (5). But in addition to the venous outflow the rate of urine discharge was also studied by this investigator. When adrenin was added to the perfusate to make a dilution of 1: 50,000 both the venous and urine flow were markedly decreased and the kidney volume as determined by an oncometer was also lessened. The dogs from which the kidneys were obtained had received large doses of morphine. It may be noted that Sollmann used solutions from twenty to one hundred times as concentrated as the adrenin solution in the adrenal veins as determined under ordinary experimental conditions.

In three perfusion experiments Pari (6) found that solutions of 1: 20,000 to 1: 100,000 caused renal vasoconstriction, but in one case a solution of 1: 500,000 caused dilatation for a few minutes followed by constriction.

Jonescu (7) recorded the effects of intravenous injections of adrenin on the blood pressure and kidney volume of rabbits. Doses which caused a moderate rise of blood pressure caused a slight dilatation of the kidneys which was followed by a marked contraction that persisted for some time after the arterial pressure had returned to normal. When smaller doses were used the kidneys showed contraction while the blood pressure remained practically unchanged. From this observation a theory was deduced that the kidney vessels have a special affinity for adrenin. It would be possible, therefore, for the adrenals by a slight continuous overactivity to set up a nephritis without any significant vascular hypertension. The theory is obviously untenable on the grounds cited. The work of Hartman (8) and that reported in the first of this series (9) accounts for the effects observed without invoking any special "affinity" of the renal vessels for adrenin. While the kidneys were contracting blood-vessels in other parts of the body were expanding, hence the systemic pressure was little affected.

In a short paper published in 1911 Froehlich (10) reported that both *l*- and *d*-suprarenin as well as "adrenalin" cause a protracted contraction of the kidneys. A more extensive investigation along the same line was reported by Ogawa (11) a year later. He used both *l*- and *d*-adrenin and synthesized *l*- and *d*-suprarenin. Instead of the oncometer method, however, he utilized perfusions to determine the effect of the drugs on the kidney vessels. Rabbit kidneys in the most sensitive preparations reacted slightly to a dilution of *l*-adrenin of 1: 20,000,000 showing a diminished outflow. In case of a solution of 1: 1,000,000

the primary effect was a sharp decrease in the rate of outflow followed by a rate above normal when the adrenin was discontinued. The augmented outflow was noted also as a secondary effect if the adrenin perfusion was continued for a relatively long period. These secondary dilatations appeared only if relatively strong solutions were used—that is, dilutions of from 1: 1,000,000 to 1: 5,000,000. In two instances primary dilatation was noted with solutions of 1: 40,000,000 and 1: 50,000,000, respectively. The same results were obtained with *d*- as with *l*-adrenin except that stronger solutions had to be used. The synthetic product also gave qualitatively similar effects. In cats and dogs the same results were obtained but the threshold was higher.

In all the foregoing reports renal vasoconstriction following the administration of adrenin is a prominent feature. In most cases, however, the doses used were probably greater than the quantity that can be discharged from the suprarenal glands in a corresponding length of time.

The evidence, so far as it goes, indicates that urine secretion follows *pari passu* the vasomotor effects produced in the kidneys by adrenin. This renders important a definite determination of the question whether the vasodilatation reported by Bardier and Frenkel and by Ogawa is a significant feature of the response to adrenin injections. If it is characteristic then adrenin diuresis such as has been described by Kleiner and Meltzer (12) may well be due, partially, at least, to local vasomotor effects in the kidneys. The suprarenal glands might then justly be brought into question as involved in "spontaneous" diuresis. The fact that vasodilatation was observed only as a secondary effect with larger doses when the adrenin would be largely destroyed or the mechanism fatigued, or as a primary effect only when very small concentrations were employed points toward this as a physiologic mechanism, since it is probable that it is only with very high dilutions that the body normally has to deal. This might be correlated with the fact observed by Kleiner and Meltzer that in order to produce diuresis adrenin must be administered as to be slowly absorbed whereas in cases in which it reaches the kidneys promptly it acts as a renal depressant (Cow, 13). In our experiments, the report of which follows, we have accordingly been especially interested in any possible dilator effects that might appear.

Technique. In general we have followed the same methods recorded in the two preceding papers of the series. The plate plethysmograph and float recorder therein described were utilized. Etherized dogs have been used exclusively in the work on the kidneys. In most

cases only plethysmograph studies were made but in a few instances venous outflow was also recorded.

Results. In this series 16 dogs were used. In the investigation of volume changes 151 injection and 5 infusion experiments were made. In determining the effects on venous outflow 14 injections and 3 infusions were given.

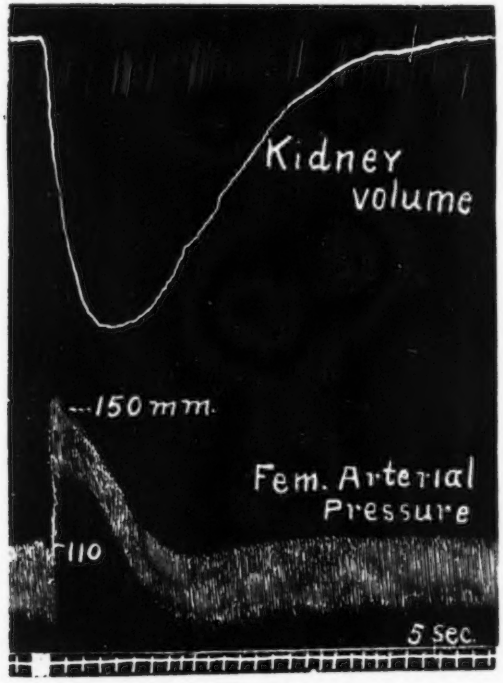

Fig. 1. Kidney contracting under influence of adrenin, 4 cc. 1: 100,000. Blood pressure from femoral artery. Time, five seconds. Dog, weight 18 kilos.

The results in the kidney were in general much the same as those in the spleen. The outstanding feature of the reaction was a sharp contraction such as that shown in figure 1. The graph reproduced is unusual, however, in one respect: neither the arterial pressure nor the organ volume show the preliminary augmentation that generally appears after adrenin injections. This augmentation which is short

lasting and inconsequential in degree is regarded as purely a passive effect due to the sudden stimulation of the heart before the outlying tissues are affected. It will be noted that the volume change outlasts for some time that of the arterial pressure. This lag is a characteristic part of the reaction picture. In various cases it was found to persist from a half to two minutes. It was usually longer towards the end of a series of experiments.

In one case only was a different type of reaction observed. This is illustrated in figure 2. After the characteristic passive preliminary dilatation as the arterial pressure rose the organ contracted. Then as arterial pressure began to fall the kidney dilated only to return to its

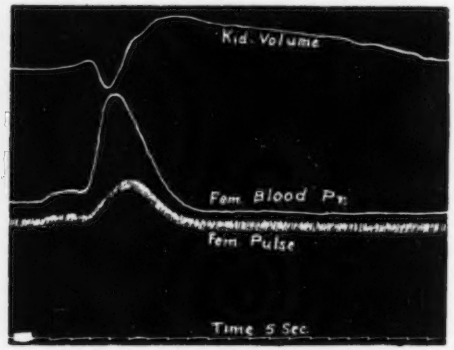

Fig. 2. Kidney expanding under influence of small dose of adrenin, 0.5 cc. 1: 200,000. Pulse and pressure from femoral artery. Time, five seconds. Dog, weight 8 kilos.

initial volume a minute after the normal blood pressure was restored. This result was observed when such doses as 0.5 cc. of 1: 100,000 were administered. When this dose was doubled the ordinary contraction of the kidney appeared and outlasted the change of blood pressure.

In no case did a pure dilatation occur such as was reported by Ogawa in two of his rabbit experiments when very small doses were used. Neither in any of our experiments was a secondary dilatation observed either during infusions or after injections or infusions such as was regarded by Bardier and Frenkel as a characteristic feature of the reaction. It should be noted, however, that only five infusion experiments were made. The results shown in figure 2 suggest that in a larger series

animals might occasionally be found which would show a dilatation of the kidneys during infusions with adrenin in high dilution. In view of the fact that infusions gave qualitatively the same results as injection in all cases observed, this possibility was not extensively investigated.

It was found that the kidneys could be held for at least ten minutes in a uniform state of contraction. Longer periods were not tried. The threshold for changes in kidney volume and for changes of blood pressure were about the same. The reactions in the kidney were qualitatively similar irrespective of whether pressor or depressor dosages were employed.

Our observations consistently support the reports of previous investigators that adrenin decreases the venous outflow from the kidney.

Our experiments as a whole do not support the theory that adrenin diuresis is due to a dilator effect of small quantities of adrenin in the kidney. On the other hand they do not definitely exclude the possibility that such may be the case in a normal unanesthetized animal. The situation as regards adrenin diuresis may well be not unlike that as regards pituitrin diuresis. From the fact that pituitrin in anesthetized animals often gives a short lasting polyuria a theory has been deduced and widely held that this substance is a diuretic agent in the normal organism, whereas, as a matter of fact it is an efficient anti-diuretic (14). In view of the evidence (a) that adrenin in doses which cause renoconstriction depresses urine formation; (b) that adrenin administered subcutaneously, and consequently, absorbed slowly, causes polyuria; and (c) that in anesthetized animals renodilatation has occasionally been reported as a result of administering adrenin in high dilutions or as a secondary reaction with larger quantities, the theory is not improbable that in normal animals adrenin in relatively small quantities causes a dilatation in the kidneys. Possibly the matter could be definitely determined by attaching metal guide strips to the poles of a kidney and then after recovery from the operation had occurred studying with a fluoroscope the renal volume reactions to adrenin when no anesthetic was used.

Marshall and Davis (15) have reported that ablation of the suprarenal glands results in depression of the functions of the kidneys even while systemic blood pressure remains normal. If, as is generally assumed, the adrenals keep the blood supplied with minute quantities of adrenin, the depression noted might in harmony with the above mentioned theory be ascribed to a deficiency of circulating adrenin, leaving the renal constrictor factors in the ascendancy. The supposition is,

however, intrinsically improbable. That renal functioning should be dependent upon minute quantities of such a dilator substance, that a hormone reaction should have been evolved to function in this purely negative way to correct a gratuitous overactivity of some other factor would seem to be a useless complication.

SUMMARY AND CONCLUSIONS

Adrenin in both depressor and pressor doses ordinarily causes contraction of the kidneys of dogs and a corresponding decrease in the venous outflow.

Instantaneous injections and slower infusions of adrenin give qualitatively similar results.

One animal gave renodilatation with smaller and renoconstrictions with larger doses.

The threshold for renal changes and blood pressure changes is about the same.

The observations as a whole do not support but also do not definitely disprove the theory that in normal animals adrenin diuresis is due to renal dilatation.

BIBLIOGRAPHY

(1) Oliver and Schaefer: Journ. Physiol., 1895, xviii, 231.
(2) Bardier et Frenkel: Journ. d. Physiol. et d. Pathol. Gén., 1899, i, 950.
(3) Gottlieb: Arch. d. exp. Pathol. u. Pharm., 1899, xliii, 286.
(4) Gioffredi: Atti. d. R. accad. med-chir. d. Neapoli, 1904, lviii, 169.
(5) Sollmann: This Journal, 1905, xiii, 246.
(6) Pari: Arch. Ital. d. Biol., 1906, xlv, 209.
(7) Jonescu: Wien. klin. Wochenschr., 1908, xxi, 513.
(8) Hartman: This Journal, 1915, xxxviii, 438.
(9) Hoskins, Gunning and Berry: Ibid., 1916, xli, 513.
(10) Froelich: Zentralbl. f. Physiol., 1911, xxv, 1.
(11) Ogawa: Arch. f. exper. Pathol. u. Pharm., 1912, lxvii, 89.
(12) Kleiner and Meltzer: Journ. Exper. Med., 1913, xviii, 190.
(13) Cow: Journ. Physiol., 1914, xlviii, 443.
(14) Motzfeldt: Journ. Exper. Med., 1917, xxv, 153.
(15) Marshall and Davis: Journ. Pharm. and Exper. Therap., 1916, viii, 525.

FURTHER OBSERVATIONS ON THE DIFFERENTIAL ACTION OF ADRENALIN

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In the course of a series of experiments performed by one of us and reported in this Journal (1), it was found that the fall in general blood pressure, which is caused by the intravenous injection of small doses of adrenalin, is not brought about by dilatation in the vessels of all parts of the body alike. In an animal in which the arteries to the abdominal organs have been clamped, injections of a standard small dose of adrenalin caused much the same fall as had previously occurred in the intact circulation. On the other hand, when the arteries to the limbs were occluded, those to the splanchnic area being intact, the reaction to the standard dose was changed from a pure fall to a rise of blood pressure, as registered from the carotid artery. In that research the only distinction drawn was the broad one as between the reactions of the "peripheral" circulation on the one hand, which included the vessels of bone, muscle, and skin, and that of the "splanchnic" circulation on the other, which, as well as comprising the vessels of the abdominal and thoracic viscera, necessarily included those of the muscles of the thorax and back. The present research was undertaken with a view to following out the subject of the differential action of adrenalin somewhat more in detail, and in the hope of arriving at some conclusion as to the mechanism involved in the vascular adjustment caused by it.

Oncometric experiments were carried out on intestine, spleen, and kidney. While our research was in progress the appearance of the paper by Hoskins, Gunning and Berry (2) made further investigation of the reactions in skin and muscle unnecessary.

In every case simultaneous records were taken of the reactions of at least two organs in response to adrenalin, since we considered it of importance to determine whether the same range of dose which caused constriction in any one abdominal organ also caused constriction in all the others, and whether the amount giving rise to dilatation in one was

necessarily the same as that which caused another to dilate. Since the reaction of any organ to a given dose may vary not only among different individuals, but also in the same individual during the period of an experiment, it is necessary to record the changes occurring in the same animal, at the same time.

The animals used were dogs and cats. In two of the experiments on the latter we injected urethane subcutaneously; in all the others the anesthetic was ether. Blood pressure was registered from the right carotid artery. Injections of adrenalin were made with a graduated syringe, the needle of which was thrust through the wall of a piece of rubber tubing fitted to a cannula, which was inserted low in the left jugular vein. The adrenalin solution used was that manufactured by Parke, Davis and Company, 1: 1,000, diluted with distilled water to the required strength immediately before use. In each experiment the first injections were made of a solution 1: 100,000; when large doses were required, as was often the case in working with dogs, in preference to injecting large quantities of distilled water into the animal's circulation, we substituted for the more dilute solution one of a strength of 1: 10,000. The duration of each injection was signaled on the record below the time marker. No special precautions were taken as to absolute uniformity in the rate of injection, but it was kept fairly constant, and was in all cases slow, as shown by the records.

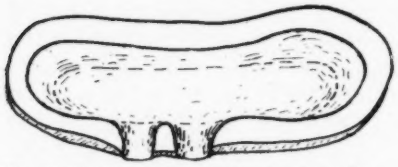

Fig. 1. Gutta percha oncometer for spleen

In some experiments we left the vagi intact; in the majority they were cut. We were unable to observe, however, any specific effect of these on the reaction of any organ to adrenalin except the familiar one of cardiac inhibition caused by large doses, with the consequent great rise in blood pressure.

The oncometers which we used for kidney and for intestine were gutta percha ones of the ordinary type, fitted with glass lids. The early experiments on the spleen were done with the same oncometers; later we had a series of special ones made. These were modelled after the shape of the spleen (see fig. 1) and were provided with two lips for stalks, separated by about 0.6 cm. in the smaller and 1.5 cm. in the larger.

As recorders in the first few experiments we used Marey's drums;

later we substituted for these bellows recorders, which have the advantage of recording volume changes without introducing alterations of pressure within the system itself. In several experiments the recorders were calibrated by injections of known volumes of air.

The pressure inside the system differed little from atmospheric; in practice we raised the pressure until the bellows were about half filled, and were thus adjusted to give maximum variations in either direction.

INTESTINE

A loop of the small intestine, about one-third of its total length, was selected, generally that immediately above the caecum, since the blood vessels there are long and form a convenient stalk. Two pairs of double ligatures were tied about its lower end, about 2 inches apart, the blood vessels supplying the piece between the ligatures tied off, and the piece of intestine removed. A similar operation was performed at the upper end of the required length, and the loop was ready to be placed in the oncometer without the necessity of further dissection, other than simply slitting the mesentery for an inch or two on either side of the stalk. Before putting the loop into the oncometer we washed out its contents with warm saline. This prevents the slow formation of gas which otherwise takes place within the lumen, and which interferes with the records of volume changes due to the circulation alone. The whole operation from the time the abdomen was opened until the intestine was put into the oncometer was not longer than fifteen or twenty minutes. During this time the intestinal loop and the other abdominal contents were kept covered with warm saline pads.

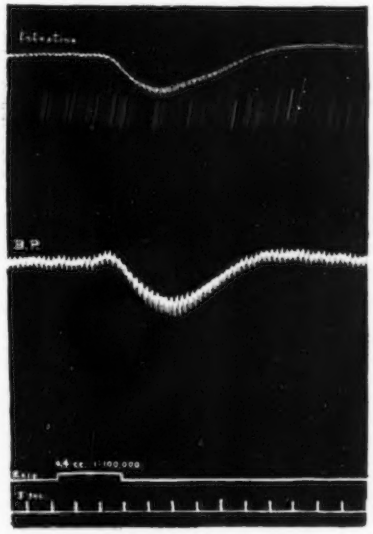

Fig. 2. Constriction of the intestine following injection of a small dose of adrenalin (0.4 cc., 1 : 100,000). Dog 14 (Reduced $\frac{1}{3}$)

In the great majority of cases, the effect of doses of adrenalin, both large and small, was to cause constriction of the intestine. In all of

these experiments small doses caused only constriction (figs. 2 and 6); as the quantity of adrenalin was increased, however, a prolonged and marked dilatation supervened on the preliminary constriction (see figs. 3 and 7). There were two exceptions, in which the least effective dose caused dilatation. These occurred during the early experiments, before we had fully realized the importance of completely removing gas from the lumen, and we consider it probable that this increase in volume of the loop was caused by the relaxation of the muscles of its walls, under the influence of the adrenalin.

The threshold for the constrictor effect was shown, in the six experiments in which it was determined, to vary within fairly wide limits, from 0.014 to 0.07 cc. adrenalin 1: 100,000 per kilogram body weight, that is, it was reached by doses such as also caused a slight fall in blood pressure. The general resemblance of these two curves, indeed, make it at first glance appear possible that the one effect may be dependent on the other, and that the constriction in the intestine may be nothing more than a decrease of blood supply to its vessels, caused by the lowering of the general blood pressure. Latent periods, duration, and degree of decrease of intestinal volume, also bear some relationship to the same changes in the general blood pressure. Closer inspection of the tables (1 and 2), however, shows clearly that this is not the case. Though the diminution in intestinal volume generally occurs several seconds after the beginning of the fall of blood pressure, this is not always the case; for instance in experiments 13, 14, and 20, the records show the intestinal decrease to precede that of the blood pressure by several seconds, and our notes, made during the course of the experiments, corroborate this as actually occurring, and not being due to a possible faulty alignment. The time of the least intestinal volume does not correspond to that of the lowest blood pressure, nor is the duration of the constriction the same as that of blood pressure fall. (See experiments 7 and 13, where it is greater, and experiments 3, 18 and 20, where it is materially less.) Above all, the constriction does not take place only when the dose is such as to cause a fall of blood pressure; constriction of the intestine occurs time and time again when the blood pressure is above and not below its normal level (see figs. 3 and 7).

The dose of adrenalin necessary to cause a dilatation of the intestine to follow on this constriction is as variable as is the threshold dose for the constriction itself. It varies from 0.04 to 0.31 cc. of a solution 1: 100,000 per kilogram in dogs, and in cats it is about 0.4 cc. The latent period of the dilatation is longer than that of the constriction,

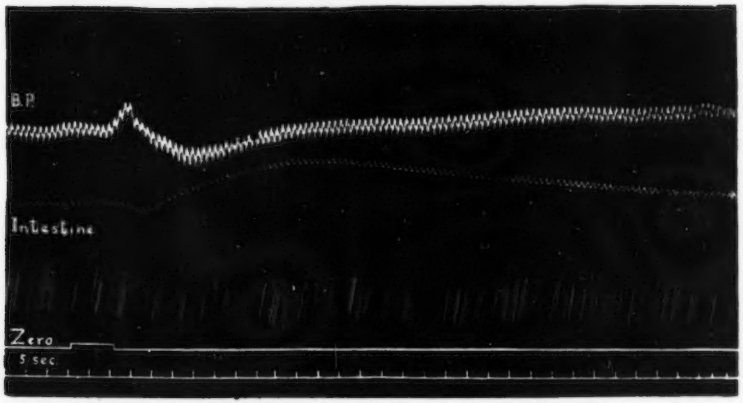

Fig. 3. Preliminary constriction followed by prolonged dilatation of the intestine, caused in the same animal as that of figure 2, by a larger dose (0.2 cc., 1 : 10,000) (Reduced ⅓)

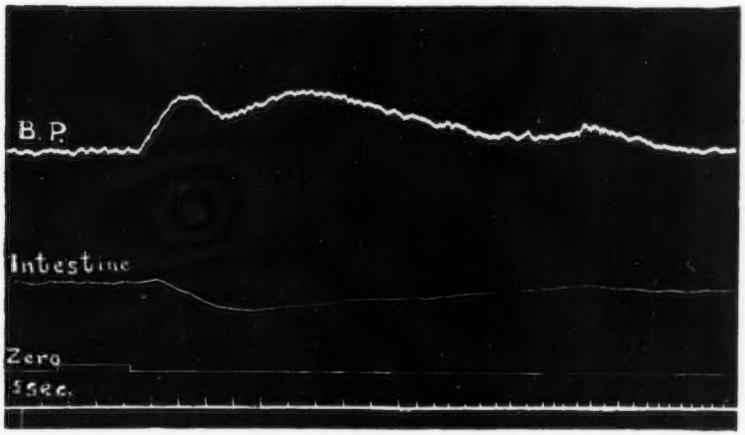

Fig. 4. Reaction of same loop of intestine as that of figures 2 and 3, to a dose of adrenalin of much the same magnitude (0.3 cc., 1 : 10,000) as that of figure 3, after the coeliac and superior mesenteric ganglia had been removed. (Reduced ⅓)

and the effect is as if the one were superimposed upon the other. As the doses are increased from the threshold dose on, the resulting constriction becomes more and more marked and its duration longer. Once the dose is great enough, however, to cause dilatation, this cuts short the first effect, with the result that the latter is reduced by from one-fourth to two-thirds of its former length. The volume change brought about by dilatation is much larger than that caused by constriction. For instance, in a dog of 25 kilograms the constriction reduced the volume of the system of the intestinal oncometer by 1 cc., while the dilatation increased it by more than 5 cc.

The observations on the resemblance of the curve of constriction to that of the general blood pressure may be applied in much the same way to this case also, for in increasing the doses of adrenalin sufficiently to cause a dilator effect in the intestine, in a few cases we crossed the threshold for pressor effect on the blood pressure. The same arguments, however, which prevented our accepting the explanation of a passive effect in constriction, are also valid in this case. The latent periods of intestinal effect are longer than those of blood pressure fall, the time of maximum dilatation never coincides with that of maximum rise of pressure, and its duration is far greater, in many instances two to three times as long (experiments 21, 22, 23, table 2). As before, too, the occurrence of the intestinal effect does not depend on the nature of the blood pressure change; we have several records which, like experiments 3 and 7, show a marked increase in intestinal volume during a fall in general blood pressure.

As a possible explanation for the occurrence of increase in the intestinal volume, after injections of doses of adrenalin above a certain size, it might be suggested that such doses are just sufficient to affect the intrinsic nervous system of the intestine and to bring about relaxation of its walls, thus permitting expansion of the blood vessels within them. To investigate this, we inserted a rubber balloon into a part of the intestine immediately adjacent to that which furnished the loop in the oncometer, injected a small quantity of air, and connected it to a small bellows recorder, which made a tracing below the oncometer record. By this it was found that injections so small as to cause only a slight fall in blood pressure were sufficient to bring about a relaxation of the intestinal wall, and that as the dose was increased no well-marked difference could be observed in the reaction of the intestinal wall to that dose which first gave dilatation in the oncometer, nor to any of the succeeding ones.

In an attempt to decide upon the origin of this dilator effect of adrenalin we severed connection between the loop of intestine involved and the central nervous system, by dissecting and removing the two coeliac ganglia and the superior mesenteric ganglion, or by cutting the splanchnic nerves. In all experiments, five in all, after this operation was performed, the dilatation by adrenalin was entirely done away with, and doses which previously had caused a preliminary constriction followed by a dilatation now gave nothing but a simple constriction of the loop (see fig. 4).

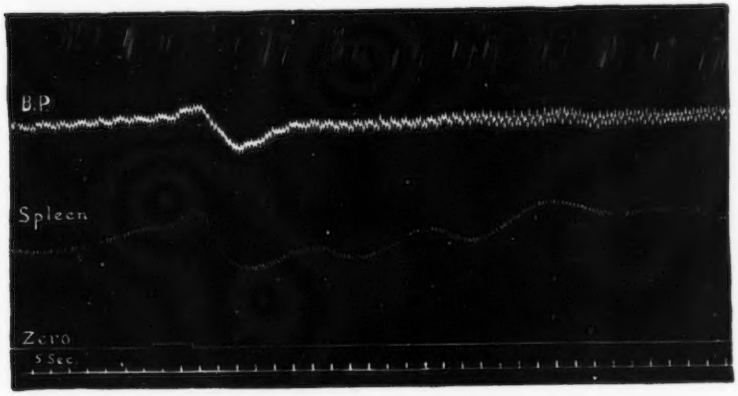

Fig. 5. Constriction in spleen, followed by a series of waves, after injection of a small dose of adrenalin (0.1 cc., 1 : 10,000). Dog 19 (Reduced ½)

THE SPLEEN

In the dissection of the spleen the gastrosplenic ligament with its numerous fat vessels was ligatured off, bit by bit, and cleared away from the neighborhood of the splenic blood vessels. In the early experiments those of the latter which supply the upper half of the spleen were also tied off. After two or three of these dissections, however, we were so dissatisfied with the appearance of the spleen under these conditions that we adopted a splenic oncometer with two lips. This enabled us to leave all vessels supplying the splenic substance intact, except sometimes that to the extreme tip of the upper end, which bound the organ too closely to allow of its being put into the oncometer. With the exception of this small piece, the spleen remained in

excellent condition, even during the course of an experiment lasting over several hours. During dissection we protected it with saline pads, and we warmed the oncometer to receive it.

Of the dogs, ten in all, which we investigated, seven showed only constriction in the spleen in response to the whole range of doses of adre-

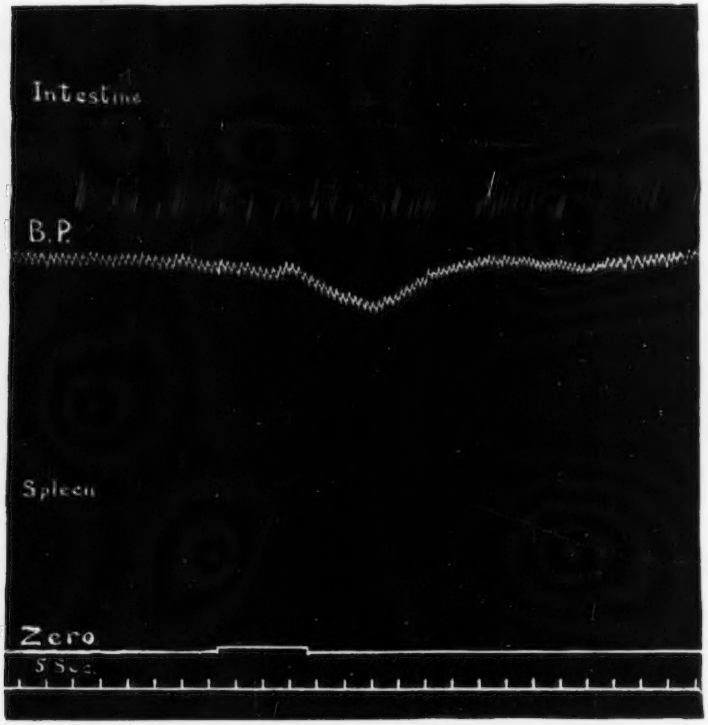

Fig. 6. Dilatation in spleen, and constriction in intestine, after small dose of adrenalin (0.3 cc., 1 : 10,000). Dog 21 (Reduced ⅓)

nalin employed. This constriction was more marked and more prolonged as the doses were increased in magnitude (see figs. 5 and 7). The three others each gave dilatation at some dose of adrenalin. Two of the three showed as a first effect dilatation with small doses; or, to speak more accurately, small doses of adrenalin set up in these

spleens (which had previously been relatively inactive) a series of waves, of which the first was in the direction of dilatation (see fig. 6). In both these organs the effect of increasing the dose was to increase the constriction in the waves at the expense of the dilatation, until large doses caused only decrease in volume. In the third of these spleens doses of adrenalin also set up series of waves, but its reaction differed

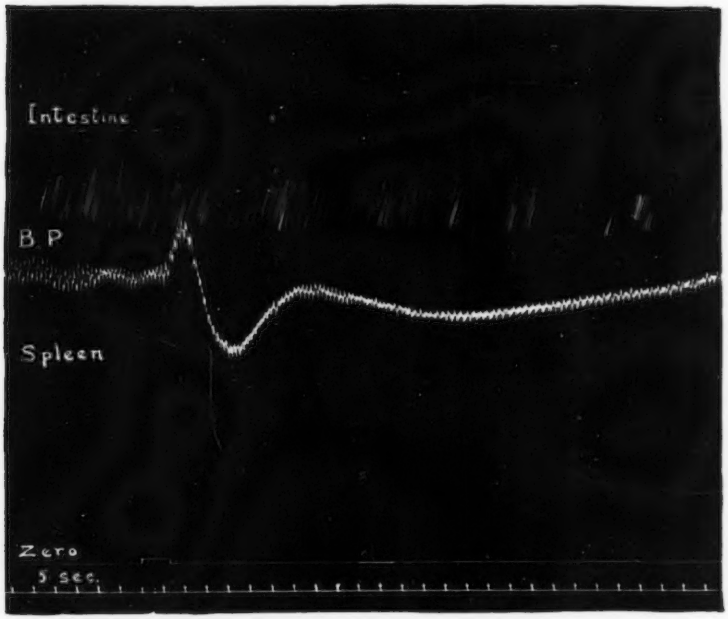

Fig. 7. Effect on same animal as that of figure 6, of a larger dose (1 cc., 1 : 10,000); slight dilatation followed by marked constriction in spleen, preliminary constriction and marked dilatation in intestine (Reduced $\frac{1}{2}$)

from that of the other two in that, on administration of relatively large doses (0.2 cc., 1 : 100,000 per kilogram) the constriction was followed by dilatation.

KIDNEY

The upper part of the ureter and the kidney vessels of one side throughout their entire length were dissected out to form a stalk. The mesentery was removed as gently as possible from the surface of the

kidney. A few of the larger veins running from it into the capsule were ligatured. During the dissection the kidney was protected as completely as possible with warm pads.

Four experiments in all were performed, two on cats and two on dogs. In every case injections of adrenalin caused constriction in the kidney (see figs. 8 and 9); with small doses of low concentration this

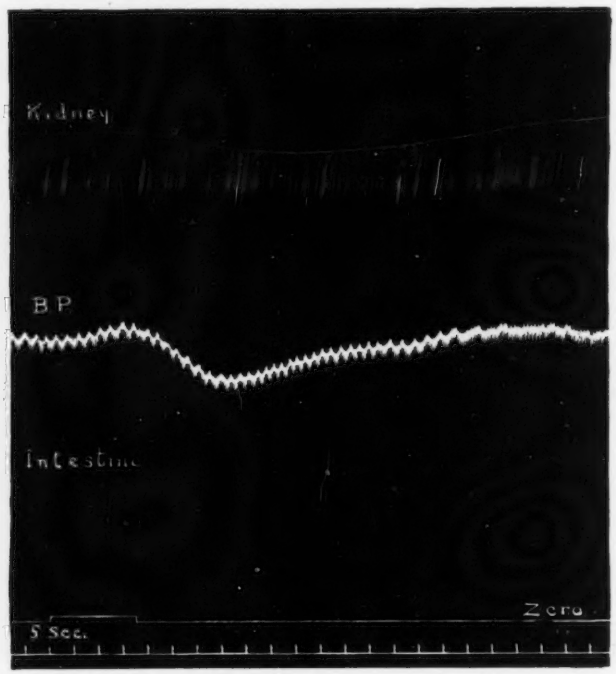

Fig. 8. Constriction of kidney after a dose of adrenalin 0.2 cc., 1 : 100,000 (Reduced $\frac{1}{2}$)

was the only effect (see fig. 8); in two cases the preliminary constriction caused by large doses (e.g., 0.32 cc. 1: 100,000 per kilogram), such as occasioned a preliminary rise followed by a fall of blood pressure, was followed by a dilatation of the organ. The curve of this dilatation was similar in form to that familiar to us in the reaction of the intestine to large doses of adrenalin. It showed a rise of

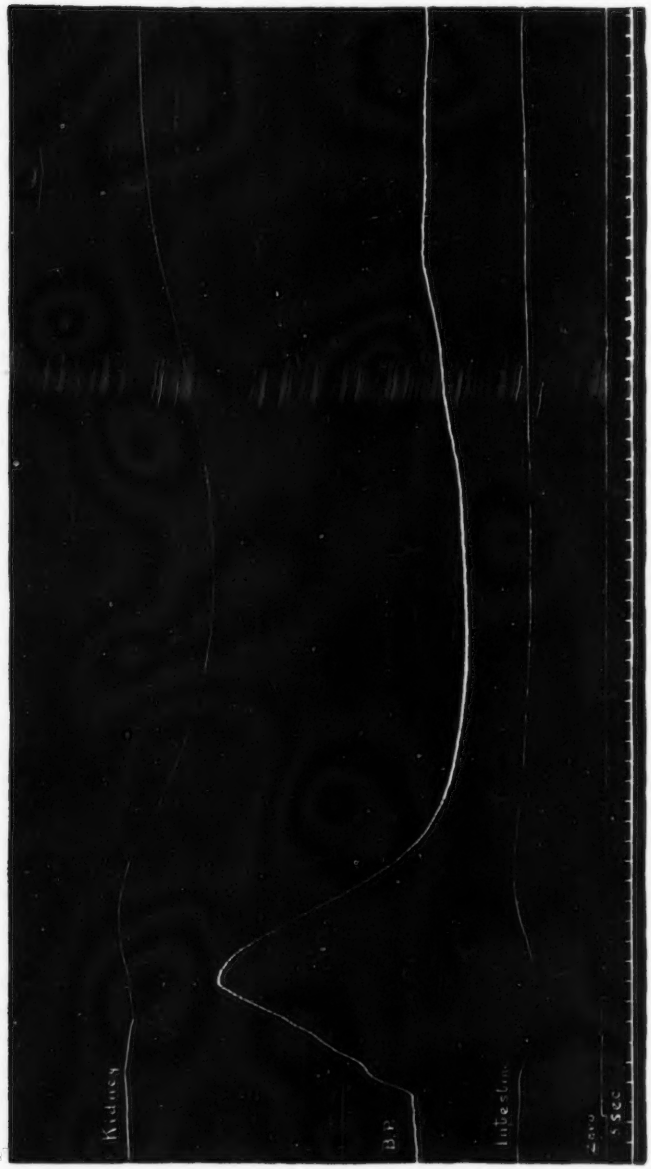

Fig. 9. Reaction of same kidney as in fig. 8, preliminary dilatation and prolonged constriction, after larger dose (0.5 cc., 1:10,000) (Reduced ½).

the lever, which only gradually returned to the base line, not before 170 to 180 seconds had elapsed, and thus continued long after the blood pressure had regained its normal level. The cause of the occurrence of this dilatation we were not able to determine.

In conclusion we wish to point out that the reactions of the various organs, though they may be of a similar nature, do not necessarily take place at the same time, nor for the same dose. Thus, for instance, in dog 23, table 2, though both kidney and intestine give constriction in response to small doses and dilatation in response to large ones, nevertheless that dose of adrenalin (0.4 cc., 1:100,000 per kilogram) which is enough to cause transition from a dilatation to a constriction in the intestine, still gives rise to nothing but a constriction in the kidney. Numerous other examples may be found by reference to that table.

That the output of adrenalin, which has been shown by the work of the last few years (3), (4), (5), to be so small during normal quiet life as to have no appreciable effect on blood pressure, is augmented during conditions of mental excitement, as well as by the asphyxia attendant on violent exercise, and by sensory stimulation, has been shown in a series of experiments by Cannon and the workers in his laboratory (6). The exact extent of this increase in secretion has not been determined, nor is it known whether it is sufficient to effect a rise rather than a fall in blood pressure. Elliott (7), in working on the secretion from the adrenal glands which is brought about by stimulation of the splanchnic nerves supplying them, has shown that in this case the quantity secreted is within the range of doses which have a depressor effect on the general blood pressure; whether this is also the case during the reflex stimulation of normal life, we are still ignorant. In any case, as shown by our experiments, the first effect of the outpouring of adrenalin during excitement must be to cause a constriction of the intestine and kidney, and generally, though not always, a similar constriction in the spleen. By this means there is brought about a shifting of the blood from these organs to the muscles, which, as Hoskins, Gunning and Berry (2) have shown, are at the same time actively dilated. If, as may prove to be the case, the output of adrenalin increases till the concentration in the arterial blood is of the order of about one-half or more that necessary to bring about a rise in blood pressure, a dilatation of the intestine, and perhaps also of the kidney, must take place, through the agency of some central mechanism, the location of which, and the source of stimulation to which, are as yet unknown.

TABLE 1

Shortening of the duration of intestinal constriction by the occurrence of dilatation

ANIMAL	WEIGHT	AMOUNT OF ADRENALIN INJECTED, CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT	LATENT PERIOD	MAGNITUDE OF CONSTRICTION	DURATION OF CONSTRICTION WHEN DILATATION IS ABSENT	DURATION OF CONSTRICTION WHEN DILATATION IS PRESENT	LATENT PERIOD	BLOOD PRESSURE CHANGE IN MILLIMETERS OF MERCURY	DURATION OF BLOOD PRESSURE CHANGE
	kgms.		*seconds*		*seconds*	*seconds*	*seconds*		*seconds*
Cat 4.....	3.15	0.06		Marked	160			Fall 136 to 110	160
		0.16		Marked		110		Fall 138 to 110	215
Cat 7.....				Small	97			175 to 184 to 142	97
				Small		13		164 to 218	63
			12	Marked		28		170 to 235 to 151	240+
Dog 14.....	7	0.03	11	Marked	32			102 to 90	33
		0.14	10	Very marked		28		99 to 87	23
		1.26*		Marked		15		96 to 180	105
Dog 20.....	9.5	0.05	12	Marked	33		11	160 to 130	60
		0.31	10	Marked		21	11	150 to 122	37
		2.08	9	Very marked		12	7	52 to 182	210
Dog.21.....	15	0.02	32	Marked	41		14	162 to 146	31
		0.67	11	Marked		29	8	106 to 122 to 86	30
		2.00	9	Marked		17	7	101 to 151	101

* All doses greater than 2 cc. adrenalin 1: 100,000 were actually injected into the animal in a 1: 10,000 concentration, but to make the dose comparable in the tables they are given in terms of 1: 100,000 dilution; 5 cc. adrenalin 1: 100,000 in the table was really 0.5 cc. adrenalin 1: 10,000 in the experiment. This applies to both tables.

TABLE 2

A comparison of the effects of adrenalin on different organs in the same individual

ANIMAL	WEIGHT	AMOUNT OF ADRENALIN INJECTED CUBIC CENTIMETERS OF 1:100,000 DILUTION PER KILOGRAM OF BODY WEIGHT	EFFECT ON INTESTINE				EFFECT ON SPLEEN		EFFECT ON KIDNEY		EFFECT ON BLOOD PRESSURE	
			Constriction		Dilatation							
			Magnitude of constriction	Duration of constriction	Magnitude of dilatation	Duration of dilatation	Magnitude of change	Duration of change	Magnitude of change	Duration of change	Change in millimeters of mercury	Duration of change
	kgms.			*seconds*		*seconds*		*seconds*		*seconds*		*seconds*
Dog 11	15	0.03	None		Small	80	Small constriction	Persistent			152 to 147	45
		0.27	None		Marked	105	Marked constriction	230			174 to 187 to 142	87
		0.67	Small	15	Very marked	120	Very marked constriction	525			161 to 175 to 150	112
Cat 13	2.9	0.07	Small	83					Marked constriction	80	132 to 140 to 109	67
		0.35	Small	172					Very marked constriction	210	80 to 92 to 68	115
		1.72	Marked	Persistent					Very marked constriction	285	106 to 213	63
Dog 21	15	0.01	No effect				Marked dilatation	50			160 to 148	20
		0.07	None		Small	130	Small dilatation followed by marked constriction				154 to 128	85
		2.00	Marked	17	Marked	210	Marked dilatation	160			102 to 152	125

Dog 22	25	0.02	No effect				Small dilatation	48			132 to 123	44
		0.20	Small	28	None		Marked constriction	Persistent			127 to 90	131
		0.60	None		Marked	285	Marked constriction followed by small dilatation	28			122 to 134 to 110	160
		1.00	Small	18	Very marked	312	Small constriction followed by persistent dilatation					
Dog 23	21.5	0.02	Barely perceptible	13		115			Marked constriction	100	143 to 121	30
		0.47	Slight	12	Small				Marked constriction	102	148 to 131	128
		0.93	None		Marked	Persistent			Marked constriction followed by dilatation	42	144 to 125 to 224	97

In investigating the dilatation occurring in various organs in the body under the influence of different concentrations of adrenalin in the blood circulating through them, we hoped to gain some light on the vexed question of the existence of dilator fibers in the sympathetic nerves to the blood vessels. The existence of these has been denied by many authorities, notably by Brodie and Dixon (8), and by Cannon and Lyman (9). On the other hand, evidence deduced from experiments of widely differing character has been brought forward in support of the theory that dilator fibers are present in the blood vessels, and are sensitive to adrenalin in solutions too dilute to stimulate the endings of the constrictor fibres. Dale's experiments on the reversal of the effect of adrenalin by ergotoxine (10), are interpreted to this effect by him. The pioneer work of Brodie and Dixon (8) on the lung already cited, and the later results of Desbouis and Langlois (11) and especially those of Enid Tribe (12) seem to point in this direction, the dilatation observed by Park (13), Elliott (17), and Cow (15), in coronary vessels, and that in vessels of other organs by Pari (16) and by Ogawa (17), in response to adrenalin offer further proof of the possibility of the existence of vaso-dilator fibres in the sympathetic system. On this subject our experiments have the value only of a negative finding, but as far as they carry us we have found no evidence of a direct stimulation of vaso-dilator endings by adrenalin, in any concentration approaching that which occurs under physiological conditions.

SUMMARY

1. Small doses of adrenalin cause constriction of the vessels of intestine, of kidney, and generally also of spleen.

2. The minimal dose necessary to produce this constriction is in much the same order of magnitude as that required to cause a fall in blood pressure, but it is not necessarily identical with it, nor is it the same for every organ in the same animal.

3. Increase of the dose of adrenalin causes in all cases marked dilatation of the intestine. This dilatation is brought about by doses materially less than those which are necessary to cause a rise in general blood pressure.

4. This dilatation in the intestine is under control of the central nervous system, and is done away with by severing connection with the central nervous system.

5. Adrenalin in the majority of cases has in all doses a constrictor

effect on the spleen; in some, minute doses cause first dilatation, which is the initial change of a series of rhythmical splenic waves.

BIBLIOGRAPHY

(1) HARTMAN: This Journal, 1915, xxxviii, 438.
(2) HOSKINS, GUNNING AND BERRY: Ibid, 1916, xli, 513.
(3) O'CONNOR: Arch. f. Exper. Path. and Pharm., 1912, lxvii, 195.
(4) STEWART: Arch. Exper. Med., 1912, xv, 547.
(5) HOSKINS AND MCCLURE: Arch. Int. Med., 1912, x, 343.
(6) CANNON AND DE LA PAZ: This Journal, 1911, xxviii, 64.
CANNON AND HOSKINS: Ibid., 1911, xxix, 274.
CANNON, SHOHL AND WRIGHT: Ibid., 1911, xxix, 280.
(7) ELLIOTT: Journ. Physiol., 1912, xliv, 376.
(8) BRODIE AND DIXON: Ibid., 1904, xxx, 476.
(9) CANNON AND LYMAN: This Journal, 1913, xxxi, 376.
(10) DALE: Journ. Physiol., 1906, xxxiv, 169.
(11) DESBOUIS AND LANGLOIS: C. R. Soc. Biol., 1912, lxxii, 674.
(12) TRIBE: Journ. Physiol., 1914, xlviii, 154.
(13) PARK: Journ. Exper. Med., 1912, xli, 532 and 538.
(14) ELLIOTT: Journ. Physiol., 1905, xxxii, 443.
(15) COW: Ibid., 1911, xlii, 125.
(16) PARI: Arch. Ital. de Biol., 1906, xlvi, 209.
(17) OGAWA: Arch. f. Exper. Path. and Pharm., 1912, lxvii, 89.

AN HYDROLYTIC STUDY OF CHITIN[1]

SERGIUS MORGULIS

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Since its discovery in 1823, chitin has aroused a great deal of interest. This substance has a very wide distribution throughout the invertebrate kingdom, particularly in the skeletons of arthropods, but the mode of its formation and its nature are still shrouded in mystery. For the solution of this problem the first and essential thing is a clear understanding of the composition of chitin. Although much attention has been given to this matter by previous investigators neither the constitution of chitin nor indeed its empirical formula may be said to be beyond dispute, and this uncertainty is due to the fact that the subject has not been approached from the proper point of view.

In general it may be said of chitin that it is characterized by extreme resistance to strong reagents. Thus it is soluble in fairly concentrated mineral acids only, and is not affected by boiling with strong alkali, in which respect it resembles glycogen. In most analytical studies of chitin, the substance was dissolved in sulphuric acid. The nature of the chitin has been argued from the products thus obtained. No attempt has ever been made—so far as I am aware—to appraise the decomposition products by a strictly quantitative method, except perhaps for the sugar which is yielded as a glucosamine.

The material for this investigation was prepared from thirty-three lobsters with the total weight of about 11 kilograms. The animals, carefully weighed, were arranged in three groups; the average weight of the lobsters in the first group was 344.1 grams, in the second group 270.2 grams and in the third 337.8 grams. The lobsters were decalcified in 10 per cent nitric acid, which for this purpose was found more effective than the hydrochloric. The decalcification was continued, the acid being frequently changed, until there was no further reaction

[1] Published with the permission of the Commissioner of Fisheries.

for calcium. The material was then washed in running water. After this preliminary treament the shell can be very easily separated from the soft parts. The large bulk of the soft tissues was therefore removed mechanically. The decalcified shells were then boiled with 20 per cent potassium hydroxide which completely digested all fatty stuff as well as every trace of meat. The hydroxide solution was renewed several times and the boiling maintained until the KOH remained colorless. The material, which at this stage is practically white, was again washed in running water. To insure complete renewal of traces of pigment it was left over night in dilute potassium permanganate, washed again in running water and bleached with sodium bisulphite. The chitin thus obtained is of a clear, snow white color. It is now washed with distilled water for several days, until tests for sulphate or chlorides are negative. The chitin is then dehydrated by treating it with several changes of alcohol, followed by several changes of dry ether. When removed from the ether, the substance dries very quickly. The material thus prepared is dazzlingly white, retains every structural detail of the original shell, and is tough as leather. The material is chemically pure chitin, leaving no ash residue on incineration. The purity of the several samples was further tested by determining its nitrogen content, as this has been found to be very constant. The final desiccation of the chitin was accomplished at a temperature below 100°C. until its weight remained unchanged. The total yield of chitin was 358.63 grams. In the first group I found 11.54 grams chitin per lobster; in the second 9.588 grams; and in the third group 11.68 grams.

AVERAGE WEIGHT OF LOBSTER	AVERAGE WEIGHT OF CHITIN	PER CENT OF CHITIN
270.2	9.588	3.08
344.2	11.54	3.35
337.8	11.68	3.45

It is evident, therefore, that the larger animals contain not merely an absolutely greater quantity of chitin, but also a relatively larger amount.

Dry chitin contains 6.39 per cent nitrogen. The elementary analysis[2] yields 6.7 per cent hydrogen and 44.94 per cent carbon. The entire composition of chitin (lobster) may therefore be presented thus:

[2] I avail myself of this opportunity to express my sincerest gratitude to Dr. P. A. Levene for the courtesy of making this analysis for me.

C	H	N	O
44.94	6.70	6.39	41.97

The empirical formula which corresponds to this elementary composition is $C_8H_{15}NO_6$. This formula may represent a monacetylglucosamine of the following constitution:

$$CH_2OH—(CHOH)_3—CHNHCH_3\ CO—CHO$$

From the fact that upon decomposition chitin yields acetic acid, which may even be detected by smell, and glucosamine, the theory has been developed that chitin represents nothing else than a polymerized acetylated glucosamine. The isolation of an acetylglucosamine from a sulphuric acid solution of chitin by Fränkel and Kelly[3] would thus seem to furnish experimental proof of the theory. We shall see later that the substance isolated by these authors probably has nothing to do with chitin, and the correspondence between the empirical formula and that for acetylated glucosamine is entirely fortuitous. The iron chain of logical arguments upon which the theory is based has always seemed to me to possess a weak link; namely, that the solution of chitin in concentrated sulphuric acid may so modify the original molecule as to completely obscure its relation to the chitin.

Following Krawkow's[4] method, I attempted to prepare chemically pure chitin by dissolving the material obtained by the above described procedure in concentrated sulphuric acid, with every precaution to prevent heating of the mixture, and precipitating it with a great excess of water. Although I made numerous trials to get chitin by precipitation—primarily because I wished to have the material in the form of a powder—I succeeded only once in getting a fine white precipitate from an hydrochloric acid solution of chitin. I will subsequently refer to this precipitated material and its probable relation to chitin. I wish merely to mention here that even in concentrated sulphuric chitin goes into solution somewhat slowly, and by the time the substance has dissolved the solution invariably gave a reduction with Fehling's, showing that the chitin was already decomposed. In searching for a method by which the chitin could be dissolved without immediately causing it to break down, it was found that much more dilute acid (60 to 80 per cent) would dissolve it as quickly as concentrated acid with the

[3] Fränkel and Kelly: Sitzungsb. der klin. Akad. d. Wissensch. in Wien, 1901, 110, Abt. 2.

[4] Krawkow: Zeitschr. f. Biol., 1892, xxix, 177.

added advantage that decomposition (as evinced by development of a brownish color) is delayed much longer. It was found still further that by raising the temperature to about 60°C with even 35 to 40 per cent acid large quantities of chitin may be easily dissolved, the solution remaining colorless for a considerable length of time.

The study of the solubility of chitin in various strengths of H_2SO_4 showed that 20 per cent of the acid is still effective and I availed myself of this weak reagent to determine the following points: (1) Will chitin dissolve in it completely if sufficient time be allowed: (2) Will this treatment cause the splitting off of the acetyl groups; (3) Will it hydrolyze the theoretical amount of glucose? With these points in mind a series of experiments was performed and the technique employed will be described here fully inasmuch as it has been followed throughout this work.

A weighed quantity of the dry chitin was put in a large Erlenmeyer flask with 200 cc. of the acid mixture. The flask was closed with a rubber stopper having four holes. Through one hole a tube passed almost to the bottom of the flask, while a short tube passed through another hole and the tubes were bent on the outside. The former was connected with the compressed air, the air bubbling through sulphuric acid and potassium hydroxide before passing through the chitin solution. The second short tube was connected to another tube reaching down to the bottom of a receiving flask containing standard sodium hydroxide, so that any volatile acid produced in the acid-chitin mixture was carried off by the air current and absorbed. Through the other two holes in the stopper were inserted a thermometer and a separatory funnel from which water could be admitted from time to time to maintain a constant volume in the flask. The flask was placed on an electric plate and the heat so regulated that the temperature never varied more than 50° to 60°C.

In the experiment which I shall describe here 1.8828 gram of chitin was used. On the basis of the structural formula quoted above this quantity of chitin should yield 0.5112 gram. CH_3COOH, 1.5336 grams. $C_6H_{12}O_6$ and 0.1193 gram nitrogen. This amount of CH_3COOH is equivalent to 85.2 cc. $\frac{N}{10}$ acetic acid. The hydrolysis was continued uninterrupted for nearly five days. At the end of that period most of the material was still undissolved, and the mixture was very turbid owing to the suspension of fine particles which have been detached mechanically through the agitation. Of the total quantity of standard alkali used 88.5 cc. $\frac{N}{10}$ were neutralized by volatile acid, driven off from

the hydrolysis mixture, as was determined by titrating with $\frac{N}{10}$ HCL, using phenolphthalein as indicator. This represents 0.531 gram. of CH_3COOH. In view of the fact that not all of the chitin had yet been dissolved, the recovery of 104 per cent of the total theoretical amount of acid made the computation on the basis of the structural formula appear erroneous.

The mixture was then filtered, the clear filtrate made up to volume and analyzed for sugar and nitrogen. This contained 0.2210 gram glucose, or 17.6 per cent.

The residue was further hydrolyzed with 40 per cent H_2SO_4. It dissolved completely within a very short time. The hydrolysis was carried on for thirty-six hours, during which time an amount of acid was produced equal to 50.1 cc $\frac{N}{10}$, or 0.3006 gram CH_3COOH. The total amount of acid thus formed was (reckoned as acetic) 0.5310 + 0.3006 = 0.8316 gram, or practically 63 per cent more than could be expected according to the formula.

In the second hydrolysis there was produced also 1.1650 grams glucose and 0.989 gram nitrogen. The total amount of glucose yielded was, therefore, 1.3860 grams (97 per cent), and that of nitrogen 0.1240 gram (103 per cent).

This and similar experiments gave weight to my skepticism towards the hypothesis that chitin is an acetylated glucosamine. It also strengthened my belief that the acetic acid detected when chitin is treated with violent reagents is not a primary hydrolytic product but rather a by-product resulting from the decomposition of the glucose molecule itself. In view of these results it was desirable to follow the different phases in the hydrolysis to get a more exact understanding, expressed in quantitative terms, of the entire process. After many trials and errors it was found that a concentration of about 35 to 40 per cent sulphuric acid (in 30 per cent the chitin is not completely dissolved) at a temperature of 50° to 60°C. are the most ideal conditions under which it is possible to carry on hydrolysis for days without causing the mixture to become much colored, and the mixture usually lacks the caramel smell. In 50 per cent sulphuric acid it is no longer safe to continue the hydrolysis very long as the mixture not merely turns a dark reddish brown, but an insoluble charred residue is formed and the whole mass smells strongly of caramel. In all such hydrolysis the amount of sugar recovered was invariably smaller than the theoretical, the glucose evidently having undergone decomposition. The production of volatile acid under the circumstances was usually very large. It is there-

fore clear that in hydrolyzing the chitin a great deal of care had to be exercised to guard against secondary decomposition. In this respect the results obtained with 40 per cent sulphuric were as nearly free from objection as is possible under the circumstances, and the account will be confined to these results exclusively.

The hydrolysis was performed by the method already described, the volatile acid being driven off by a slow but continuous current of air and absorbed in standard alkali. The hydrolytic mixture was made up to a definite volume of which aliquot portions were analysed for glucose by the Berthrand permanganate titration method. It was noted already in the early experiments that if the acid mixture was made alkaline, ammonia would distil off. When this was collected in standard acid it was discovered that the amount of ammonia nitrogen thus recovered was less than the theoretical amount of nitrogen in the chitin hydrolyzed. If the mixture was *first digested* with an excess of acid, made alkaline and distilled as is usual in Kjeldahl determinations, the ammonia nitrogen distilled off was equal to the theoretical amount. In other words, some of the nitrogenous groups were easily split off by the weak acid forming ammonium sulphate, while other groups were present in the chitin in a more stable combination and could only be set free by the complete oxidation of the molecule. It was a natural conclusion that the easily detachable nitrogenous group was the NH_2 group of the glucosamine and this was still further corroborated by the observation to be recorded later that the formation of glucose and ammonium sulphate keep pace with each other during hydrolysis. This fact suggested immediately that the empirical formula fails to convey an adequate idea of the complexity of the chitin molecule, which is certainly more intricately constituted than the adherents of the acetylated glucosamine hypothesis suspect.

In view of the significance of the results, the records of the experiments are fully reproduced in what follows:

I. August 18. 2.0339 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.

9.20 a.m. Hydrolysis started. 72.24 cc. $\frac{N}{10}$ NaOH in receiving flask.

9.50 a.m. Chitin entirely dissolved. Solution colorless.

2.00 p.m. Straw yellow color.

August 19.

9.20 a.m. Hydrolysis discontinued. Solution clear, dark yellow. Faint acid smell. Alkali in receiving flask titrated with 16.13 cc. $\frac{N}{10}$ HCL.

72.24 − 16.13 = 56.11 cc. $\frac{N}{10}$ used up.
Duration of hydrolysis, twenty-four hours.
Solution made up to 500 cc.
Two 25 cc. samples carefully neutralized. Glucose determined.
(1) 22.60
(2) 22.70

22.65 cc. $\frac{N}{10}$ KMn 0_4 used.
22.65 × 0.00663 = 0.1502 gram Cu = 0.0826 gram glucose.
0.0826 × 20 = 1.652 grams glucose.
Two 50 cc. samples made aklaline; distilled into standard acid (11.28cc. $\frac{N}{10}$).
(1) 6.42
(2) 6.33

6.38 cc. $\frac{N}{20}$ NaOH required.
11.25
3.19

8.09 cc. $\frac{N}{10}$ HCL used up.
8.09 × 10 × 0.0014 = 0.1133 gram N.

II. August 19. 2.0063 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.

9.40 a.m. Hydrolysis started. 60.20 cc. $\frac{N}{10}$ NaOH used.
After twenty-four hours. 10.05 cc. $\frac{N}{10}$ HCL required.
50.15 cc. $\frac{N}{10}$ NaOH neutralized.

August 20.
9.40 a.m. Color light yellow. 60.20 cc. $\frac{N}{10}$ NaOH used.
4.23 cc. $\frac{N}{10}$ HCL required.
55.97 cc. $\frac{N}{10}$ NaOH neutralized.

August 21.
9.40 a.m. Color dark yellow. 60.20 cc. $\frac{N}{10}$ NaOH used.
After twenty-four hours. 17.15 cc. $\frac{N}{10}$ required.
43.05 cc. $\frac{N}{10}$ NaOH neutralized.

August 22.
9.40 a.m. Color same. 60.20 cc. $\frac{N}{10}$ NaOH used.
After twenty-four hours. 20.87 cc. $\frac{N}{10}$ HCL required.
39.33 cc. $\frac{N}{10}$ NaOH neutralized.

August 23.
9.40 a.m. Hydrolysis discontinued. 188.50 cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, ninety-six hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; glucose determined.
(1) 21.40 cc.
(2) 21.30 cc.

21.35 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 21.35 = 0.1416 gram Cu = 0.0773 gram glucose.
0.0773 × 20 = 1.5460 gram glucose.
Two 50 cc. samples made alkaline; distilled into standard acid (11.28 cc. $\frac{N}{10}$).

(1) 6.60
(2) 6.70

6.65 cc. $\frac{N}{20}$ NaOH required.
11.28
3.33

7.95 cc. $\frac{N}{10}$ HCL used up.
$7.95 \times 10 \times 0.0014 = 0.1113$ gram N.
Two 50 cc. samples, digested and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 4.50
(2) 4.50

4.50 cc. $\frac{N}{20}$ NaOH required.
11.28
2.25

9.03 cc. $\frac{N}{10}$ used up.
$9.03 \times 10 \times 0.0014 = 0.1264$ gram N.

III. August 21. 2.0492 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
9.30 a.m. Hydrolysis started. 60.20 cc. $\frac{N}{10}$ NaOH used.
11.30 a.m. Slight turbidity.
4.00 p.m. Solution complete; very slight color.
9.30 p.m. Hydrolysis discontinued. 26.40 cc. $\frac{N}{10}$ HCL required in titration.
$60.20 - 26.40 = 33.80$ cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, twelve hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; glucose determined.
(1) 22.40
(2) 22.30

22.35 cc. $\frac{N}{10}$ $KMnO_4$ used.
$0.00663 \times 22.35 = 0.1482$ gram Cu $= 0.0813$ gram glucose.
$0.0813 \times 20 = 1.6260$ grams glucose.
Two 50 cc. samples made aklaline and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 6.35
(2) 6.45

6.40 cc. $\frac{N}{20}$ NaOH required.
11.28
3.20

8.08 cc. $\frac{N}{10}$ HCL used up.
$8.08 \times 10 \times 0.0014 = 0.1131$ gram N.
Two 50 cc. samples digested and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 3.85
(2) 3.90

3.88 cc. $\frac{N}{20}$ NaOH required.

11.28
1.94

9.34 cc. $\frac{N}{10}$ HCL used up.
9.34 × 10 × 0.0014 = 0.1308 gram N.

IV. August 23. 1.9990 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
4.00 p.m. Hydrolysis begun. 30.10 cc. $\frac{N}{10}$ NaOH used.
4.20 p.m. Material completely dissolved.
11.00 p.m. Hydrolysis discontinued. Solution slightly colored. Titrated with 10.20 cc. $\frac{N}{10}$ HCL.
30.10 − 10.20 = 19.90 cc. $\frac{N}{10}$ NaOH used up.
Duration of hydrolysis, seven hours.
Solution made up to 500 cc.
Two 25 cc. samples neutralized; sugar determined.
(1) 20.50
(2) 20.50

20.50 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 20.5 = 0.1359 gram Cu = 0.0738 gram glucose.
0.0738 × 20 = 1.4760 grams glucose.
Two 100 cc. samples made alkaline and distilled into standard acid (22.55 cc. $\frac{N}{10}$).
(1) 22.70
(2) 22.50

22.60 cc. $\frac{N}{10}$ NaOH required.
22.55
11.25

11.25 cc. $\frac{N}{10}$ HCL used up.
11.25 × 5 × 0.0014 = 0.0788 gram N.

V. August 23. 2.2024 grams dry chitin. 200 cc. of 40 per cent H_2SO_4. Temperature, 50° to 60°C.
4.30 p.m. Hydrolysis started. 60.20 cc. $\frac{N}{10}$ NaOH used.

August 24.
11.30 a.m. Titrated with 1.10 cc. $\frac{N}{10}$ HCL.
60.20 $\frac{N}{10}$ NaOH used.
59.10 cc. $\frac{N}{10}$ NaOH used up.

August 25.
8.30 a.m. Hydrolysis discontinued. Titrated with 19.75 cc. $\frac{N}{10}$ HCL.
60.20 − 19.75 = 40.45 cc. $\frac{N}{10}$ NaOH used up.
99.55 cc. $\frac{N}{10}$ used up.
Duration of hydrolysis, forty hours.
Solution clear, dark yellowish. Made up to 500 cc.
Two 25 cc. samples neutralized; sugar determined.
(1) 23.25
(2) 23.25

23.25 cc. $\frac{N}{10}$ $KMnO_4$ used.

0.00663 × 23.25 = 0.1541 gram Cu = 0.0850 gram glucose.
0.0850 × 20 = 1.7000 grams glucose.
Two 50 cc. samples neutralized and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 5.30
(2) 5.34

5.32 cc. $\frac{N}{20}$ NaOH required.
11.28
2.66

8.62 cc. $\frac{N}{10}$ HCL used up.
8.62 × 10 × 0.0014 = 0.1207 gram N.

VI. August 24. 1.8550 dry chitin. 100 cc. of 40 per cent H_2SO_4. Temperature, 60°C.
No aeration, flask stoppered. Material dissolved in about 45 minutes. At the end of one hour solution made up to 500 cc. On standing a slight turbidity appeared.
Two 25 cc. samples neutralized; glucose determined.
(1) 11.05
(2) 10.95

11.00 cc. $\frac{N}{10}$ $KMnO_4$ used.
0.00663 × 11 = 0.0729 gram Cu = 0.0375 gram glucose.
0.0375 × 20 = 0.7500 gram glucose.
Two 100 cc. samples neutralized and distilled into standard acid (11.28 cc. $\frac{N}{10}$).
(1) 8.10
(2) 8.20

8.15 cc. $\frac{N}{20}$ NaOH required.
11.28
4.08

7.20 cc. $\frac{N}{10}$ HCL used up.
7.20 × 10 × 0.0014 = 0.0504 gram N.

The results just presented are summarized in the following table:

CHITIN	DURATION OF HYDROLYSIS	TOTAL NITROGEN	NH_2-N RECOVERED	PER CENT OF NITROGEN RECOVERED AS NH_2-N	PER CENT OF CHITIN RECOVERED AS NH_2-N	UNRECOVERED NITROGEN PER CENT OF CHITIN	GLUCOSE (THEORETICAL)	GLUCOSE RECOVERED	PER CENT OF GLUCOSE RECOVERED	PER CENT OF CHITIN RECOVERED AS GLUCOSE	TOTAL VOLATILE ACID IN CC. $\frac{N}{10}$
gm.	*hours*	*gm.*	*gm.*				*gm.*	*gm.*			
1.8550	1	0.1185	0.0504	42.5	2.72	3.67	1.5044	0.7500	49.9	40.4	?
1.9990	7	0.1277	0.0788	61.7	3.94	2.45	1.6202	1.4760	91.1	73.8	19.90
2.0492	12	0.1309	0.1131	86.4	5.52	0.87	1.6619	1.6260	97.84	79.4	33.80
2.0339	24	0.1300	0.1133	**87.1**	**5.57**	**0.82**	1.6495	1.6520	**100.15**	**81.4**	50.10
2.2024	40	0.1407	0.1207	85.8	5.48	0.91	1.7861	1.7000	95.2	77.2	99.50
2.0063	96	0.1282	0.1113	86.8	5.55	0.84	1.6271	1.5460	95.0	77.1	188.50

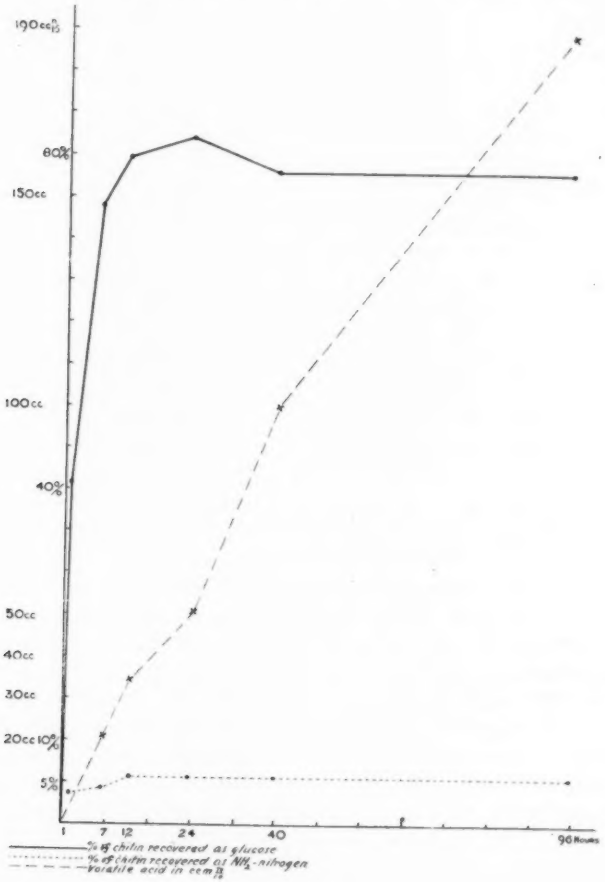
This table brings out the following points: As the duration of the hydrolysis increases, more of the NH_2 groups are split off being converted into ammonium sulphate, so that after twelve hours practically the maximum NH_2-nitrogen is obtained. This maximum represents 5.5 per cent of the chitin, while the stable nitrogen which cannot be cleaved off by the hydrolysis is about 0.8 to 0.9 per cent of the chitin. It was found that merely dissolving the chitin in 40 per cent sulphuric and allowing it to stand for one hour is sufficient to cause 49.9 per cent of the total glucose to be split off. An examination of the table will show that after 24 hours of hydrolysis a maximum yield of glucose was obtained equal to 81.4 per cent of the chitin. This coincides with the largest recovery of NH_2-nitrogen. The amount of glucose found at this stage is practically 100 per cent of the theoretical expectation. Beyond this stage further hydrolysis is accompanied by a loss of glucose and a very rapid increase of the volatile acid fraction.

The data contained in the above table are presented graphically in a series of curves (fig. 1) which bring out the various important points of this study at a glance.

We may say, therefore, that the maximum yield of NH_2-nitrogen coincides with the maximum yield of glucose and is independent of the acid formation but that if the hydrolysis is continued beyond 24 hours the great increase in the acid production is coincident with the oxidation and destruction of glucose.

If the volatile acid is the product of oxidation of glucose—in other words, if it is not a primary constituent of chitin—is it possible that it is represented by acetic acid only? It seemed to me that if the volatile acid produced in the hydrolysis was a mixture of the lower fatty acids such a finding would take away the ground from under the hypothesis that an acetyl group is present in the chitin molecule, for there would be then just as much justification—or just as little—to consider every one of those acids as a constituent part of the chitin. Owing to the difficulty of identifying various volatile acids in small quantities this side of the question has not yet been fully worked out. In one experiment the air current was passed through a solution of $AgNO_3$ and the presence of formic acid was indicated by the reduction of the silver. To verify this observation and to gain some approximate idea as to its relative amount, about 2 grams of chitin were hydrolysed in the usual way for forty-two hours and the distillate was collected in standard alkali. The amount of acid absorbed, as determined by titration, was 60.65 cc $\frac{N}{10}$. To the neutralized solution a mixture of mercuric-chloride and

sodium acetate was added; the solution was then boiled for 6 hours, care being taken to prevent evaporation. A fine crystalline precipitate of mercurous chloride was formed. This was filtered off, dried



and weighed. There was thus obtained 0.0480 gram HgCl which corresponds to 0.0047 gram HCOOH, or approximately to 1 cc. $\frac{N}{10}$. The formic acid thus formed 1.65 per cent of the total acidity produced. While these experiments must be further extended it is evident, none-

theless, that in the hydrolysis of chitin acetic acid is not the only low fatty acid liberated.

The question next to be considered is the relation to chitin of the acetylglucosamine which has been separated from acid solutions of chitin and which has the same empirical formula as the latter. The substance investigated by Fränkel and Kelly was obtained in the following manner: Chitin was dissolved in 70 per cent sulphuric acid and this was left standing for several days. When this solution was poured into an excess of water a fine powder precipitated out which on analysis proved to be monacetylglucosamine. It must be pointed out, however, that acetyldiglucosamine was likewise found (Offer[5]). In one of my experiments, already referred to, I succeeded in getting a fine precipitate by pouring a solution of chitin in hydrochloric acid into ten times its volume of distilled water. The precipitate formed very slowly, a mere turbidity appearing at first, but over night it accumulated in a fair quantity on the bottom of the beaker. It was filtered, washed free of chlorides with distilled water, then with alcohol and ether and dried. Unfortunately the elementary composition of this precipitate was not determined. But its nitrogen content throws much light on its nature especially when this is considered in conjunction with the data gained from the hydrolysis. This substance was found to contain only 5.8 per cent of nitrogen instead of 6.4 per cent which was found in the lobster material. From the hydrolysis experiments we learned already that about 0.8 per cent of chitin is in the form of a stable nitrogenous combination quite different from the remaining 5.5 per cent which represents the amino groups. It would thus seem likely that the substance I got by precipitation from the acid solution of chitin consisted of the latter moiety of the nitrogen in combination with glucose while the other nitrogenous portion remained in solution. In other words, the precipitate which was formed from chitin *was really no longer chitin*, and I believe that the substances which have been studied by other investigators were derived *from* chitin but were *not* chitin. How then is this acetylated aminosugar formed? This, too, it seems to me, is very easy to understand. The fact must be clearly borne in mind—and reference to experiment VI will substantiate this—that leaving chitin in sulphuric acid will lead quickly to its breaking up with the liberation of glucose and ammonia. Acids, too, may readily be formed through the oxidation of the glucose by the sulphuric

[5] Offer: Biochem. Zeitschr., 1907, vii, 117.

acid. The stronger the acid the more extensive and rapid may these changes be. But glucosamine and acetic combine in the presence of a dehydrating agent, and in our mixture all conditions are present (the sulphuric acid acting also as the dehydrating agent) for the recombination of these substances. This synthesis of the hydrolytic and decomposition products of the chitin molecule may account for the circumstances that the new substances thus produced may either be acetylglucosamine, or acetyldiglucosamine or diacetylglucosamine. The acid medium will hold all these in solution, but being insoluble in water they are thrown down when the acid is greatly diluted.

Having conclusively shown the fallacy of the assumption that chitin is a polymere of acetylglucosamine, a different interpretation of its composition may be attempted on the basis of the above hydrolytic studies. Referring again to the data recorded in the table, it will be noted that the two kinds of nitrogen present in chitin bear a definite ratio to each other as 1 to 7. Starting from this fundamental fact, it is clear, therefore, that chitin, whose empirical formula is $C_8H_{15}NO_6$, must be a polymere of no less than eight such groups, and its composition must therefore be $C_{64}H_{120}N_8O_{48}$. If we designate the nitrogenous portion of the chitin, the true character of which is still unknown to us, by the symbol Xn we may represent the cleavage brought about by hydrolysis by the following equations;

$$(1)\quad C_{64}H_{120}N_8O_{48} + 8H_2O =$$
$$(2)\quad 7C_6H_{13}NO_5 + C_6H_{12}O_6 + Xn + 7H_2O =$$
$$(3)\quad 7NH_3 + 7C_6H_{12}O_6 + C_6H_{12}O_6 + Xn.$$
$$\underline{\qquad\qquad\qquad\qquad}$$
$$8C_6H_{12}O_6$$

According to this chitin should yield 81.1 per cent by weight as glucose; 5.54 per cent of nitrogen in the form of ammonia (which can be distilled off directly without preliminary digestion); 0.8 per cent of nitrogen which is not split off by hydrolysis and can only be converted into ammonium sulphate by digestion. The amino nitrogen, therefore, would constitute 87.5 per cent of the total nitrogen. The agreement of the experimental findings with these theoretical expectations is truly remarkable. It reveals the great complexity of the chitin molecule which consists not merely of glucosamine but also of glucose and a nitrogenous substance of a still unknown nature That the glucose is present in the molecule in two forms is borne out by the study of the relation between the total amount of the sugar and the total NH_2-nitrogen recovered. If all the sugar were in the form of glucosamine

the ratio of N:G would be 1:12.8, but in the experiment with the maximum yield this ratio N:G is equal (0.1133:1.6520) to 1:14.6, thus proving beyond any doubt that some of the sugar does not possess the amino group.

What the nature of the unrecovered nitrogenous residue is, is still a mute point. It may very well be that it forms a nucleus around which the glucose molecules are grouped. Attempts to isolate this substance for purposes of identification and detailed study have thus far been unsuccessful, but further efforts in this direction are continued. I think that we may say with certainty that this residue cannot be of a protein nature because its nitrogen content is too low. If the reactions involved in the hydrolysis have been correctly represented in the preceding scheme—and this would seem to be thoroughly corroborated by the experimental data—this residue should have the formula $C_{16}H_{33}NO_{15}$. This formula is suggested without any intention on my part to forestall what should be based on careful study. It may serve as a guide in the research which is to follow.

SUMMARY

1. The nitrogen of the chitin molecule is partly in the form of the NH_2 group of the glucosamine, which is readily split off in hydrolysis with dilute acid, and partly in the form of a stable combination from which it can be released by digesting with concentrated sulphuric acid only. This latter portion constitutes one-eighth (12.04 to 12.45 per cent) of the total nitrogen.

2. The volatile acid produced in hydrolysis of chitin is probably a mixture of lower fatty acids. Its production is associated with a decomposition of the glucose molecule.

3. The maximum yield of glucose is about 81 per cent by weight of the chitin.

4. The evidence presented in this paper is against the accepted view that chitin is a polymerized acetylglucosamine.

5. An hypothesis is suggested, based upon results of a quantative study of the hydrolytic products, according to which chitin is composed of glucose amine, glucose and a nitrogenous moiety of still unknown nature.

THE FATE OF SUCROSE PARENTERALLY ADMINISTERED

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It has been stated by many investigators that some substances which are introduced into the organism in excessive amounts, whether they are foreign to the organism or not, can be eliminated through other channels than the kidneys, namely through the gastrointestinal tract, mammary glands, etc. Various elements such as iron, copper, strontium, barium or radium are eliminated in part, at least, through the bowel (1), (2), (3). Among organic compounds, the excretion of antipyrine, curarine, diphtheria toxin, egg protein, etc., through the intestine has been reported. (4) Many dyes, e.g., Sudan III, Biebrich scarlet, methylene blue, phenolsulphonephthalein, phenoltetrachlorphthalein, alkali blue, indigo carmine, have been reported to be eliminated into the alimentary canal, either by way of the liver or through the intestinal wall (5), (6), (7), (8), (9).

Kóssa (10) reported that in phlorhizinized hens sugar is eliminated in the intestine, the proportion of the sugar in the urine to that in the feces being as 1 : 0.3. Rösseler (11) also reported that feces of diabetic patients contained a demonstrable quantity of sugar and in an increased quantity after sugar feeding. In experimental diabetes, however, Allen (12) could find no sugar in the feces, even after feeding a large amount of dextrose. No sugar was found in the saliva of his diabetic animals. On the other hand, Naunyn (13), Brauer (7) and Woodyatt (14) were able to find reducing sugar in the bile of the animals, which were made diabetic by pancreas extirpation, piqûre or phlorhizin injection. After injecting a large quantity of sodium chloride solution intravenously into rabbits, MacCallum (15) reported that sugar is eliminated into the alimentary tract. Investigating this problem further, Fischer and Moore (16) demonstrated that rabbits which are made to secrete sugar in the urine by a sugar puncture or by intravenous injection of dextrose or sucrose solution, do not excrete sugar into their gastrointestinal tract. But when a sodium chloride solution is injected intravenously at the same time, sugar is eliminated by the small intestine. They ascribed this phenomenon to an increased permeability of the intestinal mucous membrane resulting from the salt injection. Injecting a dextrose solution alone into rabbits, intravenously, in a sufficient quantity, Kleiner proved that hyperglycemia alone can cause the sugar elimination into the intestine and stomach. The amount of sugar excreted, however, is incomparably smaller than the amount eliminated through the kidneys. A preceding double nephrectomy increases the gastrointestinal elimination of the sugar. After injecting 112 to 162 grams of dextrose in a 25 per cent solution intravenously into dogs, Grigaut and Richet

(17) also confirmed that the hyperglycemia suffices to cause sugar elimination into the gastrointestinal tract. They recovered 1.0, 3.5 and 10.2 grams of glucose, respectively in the intestinal content, while 14.0, 11.0 and 14.6 grams of glucose, respectively were found in the urine. They also demonstrated that sodium chloride and urea can be eliminated in the intestine in the same manner.

According to the reports of Mendel and Kleiner (18) and others, sucrose, introduced into the animal body parenterally, can not be recovered quantitatively in the urine. In my own previous experiments, an average of 75.5 per cent of sucrose injected was recovered in the urine. In trying to explain the fate of the rest, the question of the possible production of invertin, which has been reported by Abderhalden and others to appear in the serum, was taken up by some investigators. My previous experiments failed to demonstrate the presence of invertin in the serum. Recently, Folkmar (19) also has been unsuccessful in finding invertin in the serum after parenteral administration of sucrose. He considered that the damage of renal function, resulting from sugar injection, might have some relation to the retention of the sugar injected. Jappelli's (20) experiments suggested that part of the sucrose injected might be kept in the liver, gradually appearing in the circulation or in the bile afterwards, or might be excreted by the stomach or salivary glands into the alimentary tract.

It has already been briefly indicated that under certain circumstances other organs than the kidneys can eliminate substances which appear abnormally in the organism. At the suggestion of Prof. Lafayette B. Mendel, I have tried to find out whether sucrose parenterally administered, is in part eliminated through the liver or the intestinal mucous membrane.

METHODS

Full-grown dogs were used. In all experiments, a 10 per cent sucrose solution, sterilized by boiling, was injected into a jugular vein, sometimes very slowly (in the course of an hour), sometimes a little more rapidly (in twenty to thirty minutes). The amount of the solution injected was 150 cc. to 480 cc. The bile was collected by a biliary fistula, through a cannula in the common bile duct. After completing the experiment the bile obtained through the fistula was united with that in the gall-bladder, and this mixture was used for sugar determination. It is a very difficult problem to ascertain whether injected sucrose is eliminated through the intestinal membrane or not. The sugar may be eliminated there, but almost immediately be inverted and absorbed. In the present experiments, a loop of the small intestine was isolated between two ligatures, and a large glass cannula inserted

at each end. The upper end of the loop was a few centimeters from the beginning of the duodenum, the length of the loop varying between 22 cm. and 79 cm. The intestine was replaced in the abdominal cavity and the abdominal wall closed, only the rubber tubes connected with the cannulas being kept outside. The animal was kept warm during the experiment. Physiological saline solution, warmed to the body temperature, was introduced into the upper end of the loop, and the solution coming out from the cannula of the lower end of the loop collected. Every precaution was taken not to damage the intestinal mucous membrane and the blood vessels supplying the intestinal tract. Before the sucrose injection, the intestinal loop was washed out very gently with the saline solution (about 300 cc.), so that there was no reducing substance in the last portion. After sucrose injection, the saline solution was introduced drop by drop and the fluid coming out from the lower end of the loop analyzed for sugar. The saline solution was not permitted to be contaminated with blood. In some experiments, the blood vessels of both kidneys were ligated before sucrose injection.

For analysis sucrose in the bile or in the saline solution which was used to irrigate the intestinal loop, was first hydrolyzed by invertin. The reducing sugar, obtained, was then determined by Allihn's gravimetric method, colloidal iron being used to remove the protein and other disturbing substances. Invertin was prepared from compressed yeast, following the suggestions of Hudson. The reaction of the preparation was very slightly acid.

Concerning the occurrence of reducing sugar in the normal bile, there are many contradictory reports. In discussing those which claim the absence of sugar in normal bile, Naunyn (13) stated that a minimal amount of sugar is a quite common occurrence in the normal bile, obtained from a fistula in the common bile duct. Brauer, (7) however, demonstrated, that normal human or dog bile does not contain any reducing sugar. Woodyatt (14) reported that in his dog experiments no reducing substance was found in the bile before phlorhizin injection.

Okada (21) reported that the hydrogen ion concentration of normal dog's bile is variable within the ranges of P_H 5.34 to 6.97 in the case of bladder bile and 7.49 to 8.15 and 7.54 to 8.01 in liver bile during fasting and digestion respectively. On the other hand, the activity of the invertin is very sensitive to the hydrogen ion concentration of the medium. Following Michaelis and Davidsohn's (22) report, the optimal zone for invertin is P_H 3.67 to 5.25, the medium between P_H 3.16 and 8.30 still allowing invertin to be active. Sörensen (23)

The fate of sucrose parenterally administered

Dog	Number		I ♂	II* ♂	III ♂	IV ♂	V ♀
	Body weight		10.0 kgm.	12.5 kgm.	32.0 kg.	13.0 kgm.	8.0 kgm.
Anesthetics			Morphine (0.1 gram) + ether	Urethane (7.0 gram) + ether	Chloretone (13.0 gram + ether	Chloretone (6.0 gram) + ether	Urethane (6.0 gram) + ether
Duration of experiment			2 hrs. 20 min.	4 hrs.	3 hrs.	1 hr. 15 min.	4 hrs.
Operation			Biliary fistula	Biliary fistula + ligation of renal vessels.	Biliary fistula + intestinal loop	Biliary fistula + intestinal loop + ligation of renal vessels.	Biliary fistula + intestinal loop + ligation of renal vessels.
Sucrose injection	Amount injected		15.0 grams	22.5 grams	48.0 grams	25.0 grams	20.0 grams
	Duration of injection		20 min.	30 min.	1 hour	30 min.	30 min.
Bile	Volume	From fistula	11.4 cc.	16.0 cc.	24.5 cc.	15 cc.	4.7 cc.
		From gall bladder	10.0 cc.	9.5 cc.	36.5 cc.	16.5 cc.	5.3 cc.
	Reducing sugar, calculated as invert sugar	Before inversion	8.6 mgm.	0 mgm.	10.7 mgm.	14.8 mgm.	0 mgm.
		After inversion	24.1 mgm.	64.3 mgm.	29.3 mgm.	47.8 mgm.	29.3 mgm.

Irrigation of intestinal loop	Length of intestinal loop				22 cm.	63 cm.	79 cm.
	Volume of saline solution collected				630 cc.	310 cc.	680 cc.
	Reducing sugar, calculated as invert sugar	Before inversion			0 mgm.	33.4 mgm.	418.2 mgm.
		After inversion			45.0 mgm.	73.5 mgm.	522.0 mgm.
Urine	Volume		134 cc.	2.5 cc.	247 cc.	2.0 cc.	1.0 cc.
	Reduction	Before inversion	+	−	+	−	−
		After inversion	+++	−	+++	−	−
	Rotation	Before inversion	V° + 16.8		+ 10.9		
		After inversion	V° − 4.3		− 3.1		

* At the end of the experiment, the blood sugar content was examined by the Lewis-Benedict method. The sugar content, calculated as dextrose, was 0.08 per cent before inversion, increasing to 0.49 per cent after inversion.

ascribed P_H 4.4 to 4.6 as the optimal point for invertin, the medium between P_H 2.55 and 7.30 still allowed some activity of the ferment.

As preliminary experiments, therefore, the reducing-power of the normal bile and the activity of the invertin preparation added to the bile were examined. The bile, obtained from a normal dog, a cat and two pigs never showed any reducing power. Sucrose added to those samples of the bile was easily hydrolyzed by the invertin preparation under the experimental conditions employed. When the invertin preparation was previously boiled, or the active invertin preparation alone was added, no reducing substance was produced in the bile.

The procedure for sugar determination in the bile was as follows: After adding 5 cc. of the invertin preparation and 0.1 cc. of toluene to 10 cc. of bile, the mixture was kept $2\frac{1}{2}$ hours at 38° to 40°C. Sixty cubic centimeters of distilled water and 25 cc. of colloidal iron solution were then added and the mixture was shaken vigorously for a few minutes. Half an hour later, about 0.5 gm. of sodium sulphate was added. Seventy-five cubic centimeters of the clear and slightly yellow filtrate were used for sugar determination by Allihn's method. For control, the same procedure was always carried through with boiled invertin. When the amount of bile was not enough, less than 10 cc. of it was used for one procedure, the added substances being lessened in the same proportion. Determination of sucrose in the saline solution, which was used to irrigate the intestinal loop, was performed in the same manner. Twenty cubic centimeters of the invertin preparation were added to 100 cc. of the saline solution. Five cubic centimeters of colloidal iron solution were enough to remove protein, The control with boiled invertin was also performed. The amount of reducing sugar, both before and after inversion, was always calculated as invert sugar.

After completing the experiment, the small intestine and the ligature of the renal vessels were examined. The mucous membrane of the intestinal loop showed a slight edema in some cases. Otherwise no noteworthy changes were observed, either in the loop or in the rest of the intestinal tract. The urine obtained from the bladder at the end of the experiment was tested for sugar. When both kidneys were shut out from the circulation, the urine obtained was only a few cubic centimeters and contained no sugar either before or after inversion. When the kidney vessels were left intact the urine contained a large amount of sucrose and only a small amount of reducing sugar. When the sucrose was hydrolyzed with invertin, the urine acquired a marked levorotatory and strong reducing power. The results of the experiments are shown in the preceding table.

CONCLUSION

From the results of the present experiments, it will be seen that sucrose, injected intravenously into dogs, is eliminated very quickly through the kidneys, but at the same time, a minimal amount of it can be eliminated through the liver and the intestinal mucous membrane. The ligation of the renal vessels does not necessarily facilitate its excretion through the liver and the intestinal mucous membrane.

The permeability of the kidneys and intestinal mucous membrane for dextrose is said to be increased by parenteral administration of sodium chloride (24), (16). Phlorhizin glycosuria and glycocholia are also considered to be due to the change of the permeability of the kidneys and liver respectively.

It is not improbable that sucrose may behave similarly. Parenteral administration of sucrose, in sufficient quantities, may increase the permeability of various organs, not only for sucrose itself, but also for dextrose in the blood. This may explain why a small amount of reducing sugar preëxisted in the bile and the urine together with sucrose. In my previous work (25) already, it was noticed that reducing sugar sometimes appears in the urine after parenteral administration of sucrose, (no anesthetics were used). At that time, it was suggested that part of sucrose might be inverted somewhere in the body, so that the reducing sugar, preformed, appeared in the urine. The change of renal permeability for normal blood sugar offers another explanation. The preëxisting sugar in the saline solution collected from the intestinal loop may also be explained by change of the permeability for dextrose. At the same time, however, in this case, the activity of invertin in the intestinal juice must be considered. It can not be asserted that the irrigation of the intestinal loop, however carefully it may be performed, is absolutely harmless to the mucous membrane. It is very unlikely, however, that this is the only cause of the appearance of sucrose in the intestinal canal. On the other hand, part of the sucrose, which appeared in the intestinal loop, might be absorbed immediately, notwithstanding the constant irrigation. If such absorption did not exist, the amount of the sucrose, recovered in the loop, might be much larger.

When sucrose is injected parenterally, 20 to 30 per cent or more of the amount injected, fails to be recovered in the urine. As the amount of sucrose excreted in the alimentary tract is extremely small, this channel for sucrose excretion seems to be insufficient to explain the fate of the missing part of the sucrose injected. The question as to

whether sucrose is rendered utilizable by being inverted in its passage through the intestinal wall remains unanswered.

I desire to express my thanks to Prof. Lafayette B. Mendel, to whom I am greatly indebted for his suggestions, help and criticism; also to Prof. F. P. Underhill for his advice.

BIBLIOGRAPHY

(1) Mendel and Thacher: This Journal, 1904, xi, 5.
(2) Mendel and Sicher: This Journal, 1906, xvi, 147.
(3) Berg and Welker: Journ. Biol. Chem., 1905–06, i, 371.
(4) Langer: Zeitschr. f. exper. Path. u. Therap., 1906, iii, 691.
(5) Mendel and Daniels: Journ. Biol. Chem., 1912, xiii, 71.
(6) Salant and Bengis: Journ. Biol. Chem., 1916, xxvii, 401.
(7) Brauer: Zeitschr. f. Physiol. Chem., 1903–04, xl, 182.
(8) Abel and Rowntree: Journ. Pharm. and Exper. Therap., 1909, i, 231.
(9) Kuriyama: Journ. Biol. Chem., 1916, xxvii, 377.
(10) Kóssa: Arch. internat. de Pharm. et de Thérap., 1906, xvi, 33.
(11) Rösseler: Zeitschr. f. Heilk., 1901, xxii, Abt. f. int. Med., 302.
(12) Allen: Glycosuria and diabetes, Cambridge, 1913, 19.
(13) Naunyn: Arch. f. exper. Path. u. Pharm., 1875, iii, 157.
(14) Woodyatt: Journ. Biol. Chem., 1909–10, vii, 133.
(15) MacCallum: Univ. of California Publ., Physiol., 1903–04, i, 125.
(16) Fischer and Moore: This Journal, 1907, xix, 314.
(17) Grigaut and Richet: Compt. rend. Soc. Biol., 1912, lxxii, 143.
(18) Mendel and Kleiner: This Journal, 1910, xxvi, 396.
(19) Folkmar: Biochem. Zeitschr., 1916, lxxvi, 1.
(20) Jappelli: Jahresberiche d. Tierchem., 1905, xxxv, 79.
(21) Okada: Journ. Physiol., 1915, l, 114.
(22) Michaelis and Davidsohn: Biochem. Zeitschr., 1911, xxxv, 386.
(23) Sörensen: Biochem. Zeitschr., 1909, xxi, 131.
(24) Underhill and Closson: This Journal, 1905–06, xv, 321.
(25) Kuriyama: Journ. Biol. Chem., 1916, xxv, 521.

THE PERFUSION OF THE MAMMALIAN MEDULLA: THE EFFECT OF CARBON DIOXIDE AND OTHER SUBSTANCES ON THE RESPIRATORY AND CARDIO-VASCULAR CENTERS

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INTRODUCTION

It is generally accepted at the present time that the hydrogen ion concentration of the blood is alone concerned in the chemical regulation of respiration. Haldane (1) and his co-workers laid the foundations for this conception of the respiratory function but Winterstein (2) apparently was the first to present the facts in the form of a concrete hypothesis. Winterstein reported experiments on the perfusion of new-born rabbits which led him to the conclusion that neither oxygen-want nor carbonic acid-excess as such stimulated respiratory activity; rather the hydrogen ion concentration of the perfusate was the determining factor in the stimulation. This hypothesis has received fundamental support in the work of Hasselbalch (3), Barcroft (4) and others, and has been successfully employed as a basis for the treatment of disease and for the explanation of numerous physiological processes.

While this hypothesis has received wide application and has stimulated a renewed interest in respiratory problems, it is nevertheless open to criticism chiefly as regards its exact experimental basis, a basis which has not been strengthened by Winterstein's subsequent contribution (5). Laqueur and Verzár (6) using Winterstein's method of perfusing new-born rabbits, believed they were able to show that carbonic acid acts as a specific respiratory stimulant. The evidence which they offer, however, is not convincing. Rona and Neukirch (7), studying the behavior of isolated loops of rabbit's intestine in different solutions, found that the addition of sodium bicarbonate materially improved the rhythmic contractions of the preparation. Furthermore, they were able to show that the effect thus produced was

apparently in no wise associated with the concomitant hydrogen ion concentration of the solution. We have here an instance, therefore, in which the HCO_3 ions apparently act specifically. Consequently it has seemed advisable to put the original hypothesis as advanced by Winterstein to a new experimental test. This test was to perfuse the mammalian medulla by a method previously described (8) using blood perfusates of which the hydrogen ion concentration and carbon dioxide tension were known, and to study the effects produced by these two factors upon the activity of the respiratory center. Our results would indicate that an adequate statement of the regulation of respiratory activity cannot be fully summarized in the brief statement of Winterstein's hypothesis. We have obtained evidence that carbonic acid exerts a specific influence upon the respiratory center independent of its effect upon the hydrogen ion concentration of the blood perfusate. From these results the conclusion seems justifiable that either carbon dioxide acts as a specific stimulus or alters the irritability of the center to the normal stimulus.

EXPERIMENTAL

A number of preliminary experiments were performed which demonstrated that the method was adequate to the problem in hand. Reference to table 1 will make it clear that the respiratory center responds to changes in the blood perfusate in accordance with current ideas on this subject. These experiments show the general nature of the response. Sodium bicarbonate and sodium hydroxide depress respiratory activity; carbonic acid, hydrochloric acid, lactic acid and oxygen-want increase respiratory activity.[1] The effect upon the cardiac and vasomotor centers appears to be of the same order as that upon the respiratory center. That is to say, those substances which increase respiratory activity tend to increase the arterial blood pressure and heart-rate, and those substances which depress respiratory activity tend to decrease the arterial pressure and heart-rate. The response of the latter centers is what we should expect from our knowledge of the functional interaction of the respiratory and cardio-

[1] In the single experiment that we have carried out increased respiratory activity was very apparent when blood poorer in oxygen was perfused. The rapidity of response precludes the possibility of the formation of acid metabolic products by decreased oxidation and suggests that oxygen-want may cause increased irritability of the respiratory center. Further work along this line is contemplated.

TABLE 1

Condensed results of preliminary experiments

DATE	TEST MATERIAL	BLOOD p_H =		RESPIRATION							BLOOD PRESSURE	HEART RATE
		Control	Experiment	Amplitude in millimeters		Rate per minute		Rate × amplitude		Per cent change		
				Before	Result.	Before	Result.	Before	Result.			
June 16, 1915	NaOH			10	0	33	0	330	0	−100	>	Stopped
	HCl			5	15	15	24	75	360	380	<	◊
				10	30	3	15	30	450	1,400	◊	87 to 72
November 6, 1915	$NaHCO_3$			10	12	30	24	300	288	−4	>	◊
				7	10	33	24	231	240	4	>	◊
December 17, 1915	CO_2 5 per cent	7.6	7.4−	40	38	6	8	240	304	27	◊	◊ >
		7.6	7.4−	40	37	6	10	240	370	54	>	>
	CO_2 10 per cent	7.6	7.2	10	65	39	57	390	3,585	819	<	81 to 120
		7.6	7.2	10	25	27	30	270	750	178	◊	◊
December 21, 1915	HC	7.6	7.2	7	15	27	37	189	555	178	◊	◊
		7.6	7.2	20	20	21	21	420	420	0	◊	◊
	CO_2 10 per cent	7.6	7.2	15	30	27	30	405	900	122	◊	◊
	HCl	7.6	6.9−	20	20	18	33	360	660	83	◊	◊
January 18, 1916	HCl	7.4	7.25	10	25	57	54	570	1,350	137		
		7.4	7.25	15	35	90	75	1,350	2,625	94		
	Lactic acid	7.4	7.3	10	25	78	75	780	1,875	140		
		7.4	7.3	10	20	78	66	780	1,320	69		
		7.4	7.3	7	20	75	66	525	1,320	151		
		7.4	7.1	10	20	87	78	870	1,560	79		
		7.4	7.1	10	20	84	69	840	1,380	64		
	HCl	7.4	7.0	5	15	81	75	405	1,125	178		
		7.4	7.0	10	15	82	75	820	1,125	37		
	Lactic acid	7.4	7.3	10	15	81	81	810	1,215	50		
		7.4	7.1	5	15	63	63	315	945	201		
January 31, 1916	O_2—want, weak	7.5	7.5?	25	45	54	48	1,350	2,160	60	<	108 to 144
		7.5	7.5?	20	35	57	57	1,140	1,995	75	<	126 to 144
		7.5	7.5?	35	35	51	42	1,785	1,470	−18	<	129 to 132
	O_2—want, strong	7.5	7.5?	20	45	54	54	1,080	2,430	125	<	◊
		7.5	7.5?	20	35	57	51	1,140	1,785	57	<	◊

vascular centers. Nevertheless, the action of these several substances on the cardio-vascular centers has not been discussed and the results are, therefore, of interest.

It will be noted that the response of the cardiac and vasomotor centers is not so definite as that of the respiratory center. This may be due in part to a lesser sensitiveness of the centers themselves, but it seems more probable that the difference is primarily due to the condition of the peripheral mechanisms. In these experiments circulation is maintained in the trunk to preserve the diaphragm as a recording mechanism for respiratory activity and no effort is made to retain normal conditions as to temperature, etc., for the peripheral vasomotor system. The trunk, therefore, may be regarded as being in "shock." The cardio-motor fibers are, on the other hand, almost certain to suffer some injury in the technical procedure of inserting the perfusion cannula in the common carotid artery close to its origin on the aortic arch. It is not surprising, therefore, that cardio-motor responses should be weak and often entirely absent.

In both table 1 and table 2 the figures in the column under percentage change in respiration represent the change in the factor rate $\times$ amplitude of respiration expressed in percentage of the same factor before the experimental blood was tested. The minus sign ($-$) before the figure indicates a decrease in the factor; its absence indicates an increase in the factor. This factor is used to give an approximate indication of pulmonary ventilation. It is of significance because, as will be noted, variations of rate and amplitude do not run parallel; sometimes the one and sometimes the other determines the value rate $\times$ amplitude and sometimes one is increased while the other is decreased. But their product gives quite consistent results throughout the table. To this there are but two exceptions, namely, in the experiment of November 6, 1915 and of January 31, 1916, and in these cases the deviation from the control values is relatively small.

The necessity of using this factor (rate $\times$ amplitude) as a basis of comparing the results obtained, raises the interesting question as to whether or not sensory impulses from the lungs alone control the rate of respiration. Scott (9) has presented evidence to show that when carbon dioxide is inspired the increase in rate of respiration is wholly dependent upon vagus function; that when the vagi are sectioned and carbon dioxide is administered, the only result is an increase in depth of respiration. In the experiments here reported, the rate as well as the amplitude of respiration is variously affected. The vagi are in-

tact but the thorax is open and the lungs are being regularly expanded with artificial respiration. Vagal action cannot, therefore, be of influence in determining the respiratory rate, and the conclusion appears inevitable that variations in the blood supplying the respiratory center may determine not only the amplitude but also the rate of respiratory discharge.

In one of these preliminary experiments we used lactic acid to render the blood more acid to see if its effect differed from that of hydrochloric acid yielding the same hydrogen ion concentration. The experiment is pertinent in that lactic acid is a normal product of intermediary metab-

TABLE 2

Condensed results in comparison of CO_2 *with* HCl

DATE	TEST MATERIAL	BLOOD p_H		RESPIRATION							BLOOD PRESSURE IN MILLIMETERS
				Amplitude in millimeters		Rate		Amplitude × rate		Per cent change	
		Control	Experiment	Before	During	Before	During	Before	During		
June 15, 1916	CO_2 5 per cent	7.6	7.31	3	35	69	87	207	3,045	1,366	40
	HCl	7.6	7.31	45	40	48	75	2,160	3,000	39	5
	CO_2 5 per cent	7.6	7.31	35	55	39	84	1,365	4,620	165	25
June 21, 1916	CO_2 5 per cent	7.55	7.25	18	50	75	93	1,350	4,650	244	−8
	HCl	7.55	7.25	19	25	78	96	1,482	2,400	62	0
	CO_2 5 per cent	7.55	7.25	25	25	45	72	1,125	1,800	60	15
June 22, 1916	HCl	7.6	7.23	3	5	75	78	225	390	73	6
	HCl	7.6	7.23	5	9	78	99	390	891	128	−9
	CO_2 5 per cent	7.6	7.23	5	100	72	114	360	11,400	3,069	3
	CO_2 5 per cent	7.6	7.23	55	70	18	48	990	3,360	239	5

olism and is formed in excessive quantities under certain conditions which lead to lowering of the carbonic acid content of the blood. Under these conditions it might presumably be as efficient as carbonic acid in calling forth a respiratory response and more efficient than other acids, for example, hydrochloric. The results of this experiment indicate that there is no marked difference in the action of the two acids and since hydrochloric acid was somewhat more convenient, we used it in the subsequent experiments designed to investigate the specificity of carbonic acid as a respiratory stimulant.

Our experiments were directed primarily to a comparison of the respiratory response elicited by blood, the hydrogen ion concentration

of which was altered to the same degree by the addition of carbon dioxide and hydrochloric acid. To this end we used as a control, blood which had been shaken to remove the excess of carbon dioxide and observed the physiological reaction produced by the substitution of bloods with higher hydrogen ion concentration.

In this procedure a number of precautions had to be observed. (1) *General character of the several perfusates.* To insure the utmost similarity in regard to possible unknown factors, the defibrinated blood was collected, mixed and aerated in a porcelain-lined vessel after which it was subdivided in clean stoppered flasks. These samples were then submitted to the appropriate change of hydrogen ion concentration and placed in a constant temperature water-bath until used. (2) *Temperature.* Variations in temperature doubtless influence the behavior of the medullary centers. To guard against possible errors due to this factor, the sample, the response to which was next to be investigated, was placed in the substitution reservoir of the apparatus some ten minutes before being used so that its temperature, if not actually that of the control in circulation, was at least constant as compared with any other similar sample investigated in the same experiment. (3) *Change in irritability of the respiratory center.* The medullary centers undoubtedly lose in functional capacity in the course of perfusion and this rate of loss is not comparable in different experiments. It is evident, therefore, that the comparison of the response produced by two stimuli so closely alike as, for example, carbon dioxide and hydrochloric acid bloods, is unsafe unless they are both tested in the same experiment. And even then the progressive loss of irritability may establish conditions such that the test of the second specimen of blood is, for purposes of comparison, invalid. If, however, the perfusion of carbon dioxide blood produces the greater increase of respiratory activity when tested *after* as well as *before* hydrochloric acid, we may believe that the carbon dioxide blood is the more effective stimulus. This we were able to show in the present experiments.

PREPARATION OF PERFUSATES

The defibrinated blood used as control was agitated in air until its hydrogen ion concentration had reached a minimum. One portion of this blood was placed in a flask and agitated while being exposed to an atmosphere of 5 per cent carbon dioxide in oxygen. The hydrogen ion concentration of this sample was determined independently by two

of us using the indicator and electrical methods. A second portion was brought to the same reaction by treating with hydrochloric acid and shaking to remove the excess of carbon dioxide liberated. In this way two specimens of blood were prepared; one in equilibrium with a high tension of CO_2 (5 per cent of an atmosphere) and therefore containing a considerable quantity of free carbonic acid and a relatively high concentration of total carbonate; the other in equilibrium with a low tension of CO_2 (0.04 per cent of an atmosphere) and containing relatively less carbonic acid and total carbonate but with the same hydrogen ion concentration as the former.

The reactions of both specimens were determined by the colorimetric and the electrical methods. The former could be used advantageously to obtain approximate results in a short time, while the latter method furnished the more trustworthy results especially with the bloods containing carbonic acid. These determinations were carried out at room temperature, using the improved Hasselbalch hydrogen electrode, McClendon potentiometer and a recently standardized Weston cell. A portion of the blood specimen was placed in the electrode to establish CO_2-equilibrium with the hydrogen gas and then replaced with a second portion on which the determination was made in the usual manner.

Three experiments comparing the efficacy of carbonic acid and hydrochloric acid bloods were performed. The results are presented in table 2. The headings used in this table are the same as those used in table 1 and require no further consideration. We wish, however, to draw particular attention to the figures in the column under percentage change of respiration. These figures bring out forcefully the difference in response to the two experimental bloods. Carbonic acid blood when used before as well as after hydrochloric acid blood produces much the greater effect. The apparent exception to this statement in the second test of carbonic acid blood in the experiment of June 21 may, we believe, be properly explained by a progressive deterioration of functional activity in the center. In this case the response is at least as great as it was in the preceding test with hydrochloric acid blood. The influence of such a progressive loss of function was considered earlier in the paper and we do not believe that our conclusions are weakened by one instance in which carbonic acid blood failed to elicit a greater response than that produced by hydrochloric acid blood tested previously.

Portions of the graphic record of one of these experiments are pro-

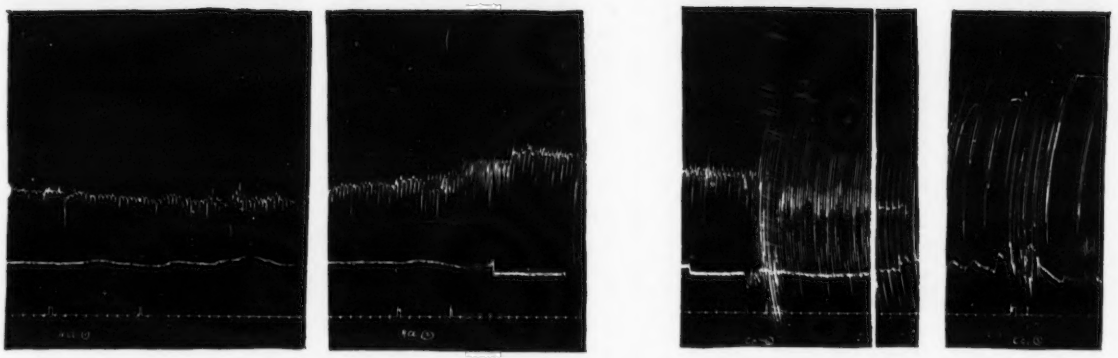

Fig. 1. Portion of graphic record obtained in experiment of June 22, 1916. Upper record, respiratory movements of epigastrium. Middle record, blood pressure recorded with mercury manometer. Lower record, zero blood pressure with time in two second periods. The two breaks in this line indicate in each section of the record the beginning and completion of the perfusion with the experimental blood. The reaction of the normal blood was $P_H = 7.55$; that of the hydrochloric acid blood $P_H = 7.25$ and that of the carbon dioxide blood $P_H = 7.25$. Note the relatively slight effect produced by the hydrochloric acid blood, although it was allowed to act for a longer time when the medullary centers were presumably more irritable. Note also the shorter latency in the reaction with carbon dioxide blood.

duced in figure 1, the protocol of which may be given as an example of all.

Experiment of June 22, 1916. Comparison of effects of blood brought to the same hydrogen ion concentration with CO_2 and HCl on dog's medullary centers. Large dog bled several times after intravenous injections of warm Ringer's solution. Total volume of defibrinated blood thus diluted about 3000 cc. While still warm the blood was aerated by rapid stirring in a large vessel for half an hour to liberate excess of CO_2. Four hundred cubic centimeters were submitted to an atmosphere of CO_2 5 per cent + O_2 95 per cent by bubbling the gas through a trapped flask containing the blood for about fifteen minutes. Two hundred cubic centimeters were treated with M/7 HCl in 0.4 per cent NaCl and the blood shaken until the hydrogen ion concentration ceased to diminish, i.e., until the excess of free carbon dioxide was removed. The reactions of the three specimens were:

	INDICATOR METHOD	ELECTRICAL METHOD
Normal blood	p_H = 7.6+	p_H = 7.55
CO_2 blood	7.25	7.22
HCl blood	7.25	7.22
Normal blood after repeated perfusion		7.51

Blood in stoppered flasks placed in water bath at 36°C.

The experimental animal was operated under chloretone anesthesia. The perfusion was at 80 mm. Hg, and 37°C. The venous outflow was about 180 cc. per minute (30 cc. in 8.5 seconds). Probably the normal blood will tend toward an acid reaction as the rapidity of flow made it technically difficult to segregate the experimental blood. During perfusion of the experimental bloods they were of necessity partly exposed to room atmosphere. This will not affect the HCl blood but may result in lowering the hydrogen ion concentration of the CO_2 blood by diffusion of the gas.

Inspection of the figure shows, furthermore, as indicated in the elapsed time between the breaks on the time record that the hydrochloric acid blood was perfused for periods of eighteen and ten seconds without eliciting a sharp reaction in either case, while the perfusion of CO_2 blood for two periods of four seconds each produced not only a much more decided reaction but one which exhibited itself very much more promptly. The fact is significant as further indicating the greater efficacy of carbon dioxide. The other experiments in this group showed this sharp response to carbonic acid blood to a lesser degree probably because the rate of perfusion was slower.

DISCUSSION

The experiments thus outlined appear to prove conclusively that blood with a comparatively high tension of carbon dioxide causes a greater stimulation of the respiratory center than does blood with a lower tension of carbon dioxide but with the same hydrogen ion concentration. The method of stimulation is unknown though several theoretical considerations are of interest.

The reactions of the bloods were within physiological limits. As the bloods with higher hydrogen ion concentration were still alkaline, the diffusion of free acids other than carbonic may be considered negligible. The similarity in the results obtained by the use of bloods to which either hydrochloric or lactic acids were added suggests that there is no appreciable difference in the diffusion of the two anions. As it would seem that lactic acid ions could easily penetrate the respiratory center on account of their great diffusibility the above observation is opposed to the conception that hydrochloric acid ions elicit a relatively small response because of their failure to readily diffuse into the same cells. The variations in the concentration of the salts in the perfusates, incident to the alteration in the reaction of the blood do not have an appreciable inhibiting action on the respiratory center, as unpublished experiments have shown. The cause for the smaller stimulation by the bloods to which hydrochloric acid was added (and the excess of carbon dioxide shaken out) must therefore be due to the decreased concentration of carbon dioxide or carbonic acid.

As the tendency for diffusion of carbon dioxide from the cells into the blood depends on the difference in the tension of carbon dioxide of the two systems, the higher the carbon dioxide content of the circulating blood, the slower the diffusion from the cells and the higher the concentration in the cells when a new equilibrium is established. The tension of carbon dioxide in the respiratory center would presumably, therefore, be higher in our experiments in which blood with a high tension of carbon dioxide was employed and, vice versa, low when blood with low carbon dioxide concentration was used. It would seem that the hydrogen ion concentration of the two systems would likewise maintain an equilibrium. If this is true, the hydrogen ion concentration in the center would be the same in both series of experiments because the reaction of the two bloods was the same. The greater activity produced by the bloods containing the high tension of carbon dioxide must therefore be due to some specific action of the carbon dioxide.

Whether carbon dioxide as such stimulates the respiratory center or whether variations in the carbon dioxide concentration alter the irritability of the respiratory center can hardly be demonstrated. If we are to adopt an explanation for the results obtained in these experiments, the most satisfactory point of view would seem to be that the hydrogen ion concentration of the environment of the respiratory center is its effective stimulus but that the irritability of the center to this stimulus may vary and be influenced by many factors. The normal irritability of the respiratory center is doubtless the summation of a number of effects including those produced by carbonic acid, oxygen and various ions as well as changes in metabolic activity, the nature of which is not understood.

CONCLUSIONS

1. An increase in alkalinity of the perfusing blood (produced by adding sodium bicarbonate or sodium hydroxide) tends to depress the medullary centers.

2. A decrease in alkalinity of the perfusing blood (produced by adding hydrochloric acid, lactic acid or carbon dioxide) tends to stimulate these centers.

3. A specimen of blood containing a high tension of carbon dioxide causes greater activity of the respiratory center than another specimen of the same hydrogen ion concentration but with a low tension of carbon dioxide. Carbonic acid thus acts specifically upon the respiratory center.

BIBLIOGRAPHY

(1) Haldane: Organism and environment, New Haven, 1917.
(2) Winterstein: Pflüger's Arch., 1911, cxxxviii, 167.
(3) Hasselbalch: Biochem. Zeitschr., 1912, xlvi, 403.
(4) Barcroft: The respiratory function of the blood, Cambridge, 1914.
(5) Winterstein: Biochem. Zeitschr., 1915, lxx, 45.
(6) Lacqueur and Verzár: Pflüger's Arch., 1912, cxliii, 395.
(7) Rona and Neukirch: Pflüger's Arch., 1912, cxlviii, 273.
(8) Hooker: This Journal, 1915, xxxviii, 200.
(9) Scott: Journ. Physiol., 1908, xxxvii, 301.